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SOIL SCIENCE

Contents for November, 1921

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INOCULATED LEGUMES AS NITROGENOUS FERTILIZERS

P. E. BROWN AND J. H. STALLINGS

Iowa State College

Received for publication March 16, 1921

The beneficial effects of the growth of legumes on the yields of subsequent crops has been known for centuries. Many explanations were suggested from time to time to account for this peculiar influence of leguminous crops in comparison with non-legumes, but none proved very tenable. It was not until the eighties of the last century that an adequate explanation was offered. The discovery, at that time, of the ability of certain bacteria when growing in nodules on the roots of legumes, to fix the free nitrogen of the atmosphere and furnish it to the plant, served to show that legumes benefit the soil mainly because, when inoculated, they increase the nitrogen supply available for succeeding crops.

This discovery also provided the explanation for many peculiar occurrences which had been noted in the growth of legumes. The ability of such crops to grow without nitrogen was found to depend upon the presence of the proper bacteria and the reason for the failure of crops in some cases and the successful growth in others under apparently similar conditions of low nitrogen supply, became evident. The large benefits occurring in some cases from the use of legumes and the absence of effect in other instances also was explained. The cause of the beneficial effect on clover of spreading soil from an old cultivated field over newly broken land was found to be due to the bacteria introduced. In short, the "abnormal behavior" of legumes, noted so many times in investigational work and in practice, was easily explainable when viewed from a bacterial standpoint.

Following this discovery of the symbiotic relation between bacteria and legumes, a large amount of investigational work began and has continued to the present time. The original experiment itself has been checked by numerous investigations and the accuracy of the conclusions drawn has been satisfactorily proven. The subject of soil inoculation has received much attention, and has led to the very practical conclusion that if legumes are to be successfully grown and well inoculated the proper bacteria must be supplied by inoculation in all cases where they are not present.

Cross-inoculation, the grouping of the bacteria from different legumes into classes, the preparation of highly efficient or virulent cultures of organisms, the non-symbiotic nitrogen-fixing power of the bacteria, the relation of the symbiotic process to the nitrogen content of the soil, the length of time during which the

organisms remain alive and vigorous in the soil and the isolation of the bacteria from the soil, are some of the more or less technical phases of the subject which have received attention. From the practical standpoint the problem of chief interest has been the relation of the growth of inoculated legumes to soil fertility and particularly to permanent agriculture. Secondary to this has been the problem of the proper soil conditions for the best growth and inoculation of legumes.

The need for a return of nitrogen to the soil in an amount equal to that removed by crops is a recognized fact in all systems of permanent fertility. This return may be accomplished by the use of artificial or natural nitrogen carriers. Commercial nitrogenous fertilizers are expensive and if the nitrogen of the atmosphere can be utilized by the growing of inoculated legumes, the nitrogen supply in the soil can be kept up much more economically. Furthermore, the legumes not only supply nitrogen but also add organic matter to the soil and thus have a double value. Practically all systems of farming at the present time therefore, include the use of legumes in the rotation and it is quite generally accepted that *by proper handling of these crops* either as a definite part of the rotation or as green manures, the nitrogen supply in the soil may be very largely maintained without the use or by only small additions of commercial nitrogenous fertilizers. It is admitted that such materials may prove profitable in some cases for special soil and crop conditions but on the *average* soil for general farm crops they are considered as supplements to inoculated legumes.

While it is commonly believed, therefore, that inoculated legumes add nitrogen to the soil and that these crops are very valuable nitrogenous fertilizers, it is a matter of considerable interest to note that there is very little definite knowledge regarding the actual amount of nitrogen which legumes add to the soil. There has been much theorizing, and many assumptions have been made but without any very definite background of fact for field conditions. A pamphlet published several years ago (7) calls attention very strikingly to the dearth of accurate information on this important point.

It is obvious that if we are to depend upon legumes to supply most of the nitrogen needed for the permanent fertility of soils, considerable data must be secured by extensive experiments which will show the actual amount of nitrogen which legumes take from the atmosphere or rather the relative amounts secured from the atmosphere and from the soil under a wide range of soil conditions, particularly as regards nitrogen content. It is probably true, as is now believed, that on soils rich in nitrogen less of that element is taken from the air than is the case on soils low in nitrogen. It is probably true also that it is more difficult to secure inoculation of legumes on rich soils than on soils low in nitrogen and organic matter. Some information has been secured regarding the relative nitrogen content of the tops and roots of legumes, but further data along this line should be secured under control conditions with different legumes and on various soils, if definite recommendations,

regarding the proper handling of legumes to secure the best effects on soils, are to be made. Again, practice in this matter is based upon assumptions rather than upon the results of scientific experiments.

The nitrogen problem in permanent agriculture cannot be solved until there is much further experimental work carried out along the lines indicated. Then and only then will it be possible to make definite and complete recommendations which will stand the test of long-continued practice.

The work reported in the following pages was planned to secure preliminary information on some of the problems mentioned above, and while the results are of course far from complete, they are presented here inasmuch as they indicate quite clearly the nitrogen fixed by two common inoculated legumes on two extensive soil types in this state, and as they show also the distribution of nitrogen between the tops and roots of these legumes, at various stages of growth, under control conditions.

HISTORICAL

It is not necessary to give an extended bibliography of the investigations dealing with the symbiotic fixation of nitrogen or rhizofication, as very complete lists of publications have been given in reports on various phases of the subject. It seems desirable, however, to review briefly the results of previous experiments which throw any light on the particular problems studied in this work. The references given are only those which contain information on the amount of nitrogen fixed by inoculated legumes as determined by the relative nitrogen content of inoculated and uninoculated plants, and on the relative nitrogen content of the tops and roots of legumes.

Hopkins (22) suggests the comparison of the nitrogen content of inoculated and uninoculated plants as a method of calculating the nitrogen taken from the air, and assumes that it gives correct results. He also suggests that a comparison of the total nitrogen content of a non-leguminous crop with that of a crop of infected legume plants grown at the same time on the same soil will give practically accurate information on the subject. The latter method can hardly be considered satisfactory for obvious reasons and there are objections to the former, but at least, it may serve as an indication of what may be expected from more exact tests.

Experiments at Rothamsted (42) have given evidence of increased nitrogen in soils from the growth of inoculated legumes. For example, soil from a field where clover was grown showed 0.156 per cent of nitrogen while where barley was grown the content was 0.142 per cent to a depth of 9 inches. This would amount to 350 pounds per acre of 2,500,000 pounds of soil.

Aeby (1) grew peas in a poor soil and in a rich soil and found a fixation of 1.976 gm. of nitrogen per 4 kgm. of soil in the rich soil and 2.759 gm. in the poor soil.

Duggar (11) experimenting with crimson clover and hairy vetch found a fixation of 98.5 pounds of nitrogen per acre in the one case and 139.4 pounds

per acre in the other, calculating the nitrogen in the entire crop including the roots and stubble. With both these crops the yields when inoculation was not practiced were very small.

Experiments on alfalfa in Illinois reported by Hopkins (20) give results in the field tests which show a fixation of about 40 pounds of nitrogen per acre from the air by bacteria—in one crop, on the untreated soil. When lime was added a further slight increase was obtained and with lime and phosphorus, a gain of over 53 pounds was secured. The total fixed for the season was calculated at 172 pounds on the unfertilized plots and 252 pounds on the plots receiving lime and phosphorus.

In pot tests with alfalfa reported in the same bulletin, the nitrogen fixed by bacteria in ordinary soil ranged from 7 to 90 pounds per acre. The average fixation in the unfertilized pots amounted to about 40 pounds. This amount was increased in some cases on the fertilized soils but where nitrogen was supplied the fixation was reduced. The largest amount was fixed when lime, phosphorus, and potassium were applied.

Later experiments of Hopkins (21) with cowpeas seeded as a catch crop after oats on land heavily cropped to corn and oats until nitrogen was the limiting factor of growth, showed that as an average of the results of the analyses of ten immature plants on each of six plots, three inoculated and three uninoculated, 73 per cent of the nitrogen in the inoculated plants came from the air. They showed also a fixation of 12.9 cgm. of nitrogen per plant.

Inoculation experiments with alfalfa at New Jersey (8) showed a 40 per cent gain in two cuttings by inoculating with a soil infusion. A smaller increase was given when soil was used as the inoculum. The crop was not analyzed but assuming the same per cent of nitrogen in both inoculated and uninoculated plants, a large nitrogen fixation was apparent.

Experiments by Shutt (34) in Canada showed a gain in nitrogen in pot tests of 0.0065 per cent, or 130 pounds per acre of 2,000,000 pounds of surface soil, from the growing and turning under of mammoth clover for two successive seasons. In plot tests for two seasons, with two cuttings removed, and all residues returned, there was an increase of 0.0143 per cent, or 286 pounds per acre, an annual fixation of 143 pounds. In later work by the same author (35) the growing of clover on a sandy soil for 6 years was found to increase the nitrogen content of the soil to the amount of 375 pounds per acre.

Nobbe and Richter (28) in one experiment found that 93 per cent of the nitrogen in vetch was fixed from the atmosphere. In a later test, 96 per cent was found to be fixed. Only the nitrogen in the tops of the plants was determined in this work. A gradual increase in the nitrogen fixed occurred up to the maturity of the plants. Tests with additions of varying amounts of nitrogen added showed a decrease in the nitrogen fixed, the decrease becoming greater with the larger additions of nitrates.

Smith and Robison (36) found 33 per cent of the nitrogen in soybeans fixed by bacteria, and with cowpeas 15 per cent of the nitrogen was secured from the air, In one case the nitrogen fixed amounted to 37 pounds per acre and in the other case to 21 pounds per acre. In two other tests with areas 12 feet square, the soybeans (tops only) took 44 per cent and 32 per cent of their nitrogen from the air, showing a fixation amounting to 283 pounds and 134 pounds of nitrogen per acre, respectively.

Studies with soybeans by Woll and Olson (45) at Wisconsin, showed that when grown on a rich soil, 14 per cent of the nitrogen in well inoculated plants came from the atmosphere. The amount of nitrogen fixed amounted to 16 pounds per acre.

In field tests of various commercial cultures for the inoculation of legumes, Lipman (23) found a fixation of 13 pounds of nitrogen per acre in the case of cowpeas. With alfalfa, a fixation of 15 pounds of nitrogen was found when soil was used for inoculating and 35 pounds when a commercial culture was employed, lime being applied to the soil in both cases. In tests with cowpeas, soybeans and bush limas in rows, there were considerable increases in the nitrogen fixed as indicated by the total nitrogen in the grain.

Alway and Pinckney (4) found a fixation of 92 per cent of the nitrogen in alfalfa plants through inoculation. The fixation amounted to 0.004107 gm. per plant.

Alway and Bishop (3) report no marked difference between the amounts of nitrogen in soils from an alfalfa field and from a corn field. Later work at Kansas by Swanson (38) and by Swanson and Latshaw (39) shows that the growing of alfalfa for 20 or 30 years did not add to the nitrogen in the soil but neither did it reduce it to any extent. Where grain had been grown continuously the soil was 21.6 per cent lower in nitrogen than under alfalfa but in the latter case there was 14.3 per cent less nitrogen than in the native sod.

Hartwell and Pember (19) determined the gain in nitrogen in a pot experiment with different legumes over a 5-year period. Cowpeas and soybeans were grown each summer and vetch was grown in the pots each winter and turned under in the soil. The approximate net gain in the presence of these crops was 1 ton of nitrogen per acre, seven-tenths of which was contained in the 25 tons of moisture-free summer crops removed and the remainder in the soil.

Lyon and Bizzel (27) grew alfalfa and timothy for 6 years and then planted corn and oats. The corn yielded 15 bushels more on the alfalfa soil while the oats showed practically no difference. The alfalfa soil contained 0.01 per cent more nitrogen than that under timothy and this would amount to a fixation of 200 pounds per acre of 2,000,000 pounds of surface soil.

Arny and Thatcher (5) studying different methods of inoculating alfalfa, found in two field tests on the Minnesota University Farm, a fixation of 18 pounds of nitrogen per acre, as an average of all methods. In another test

a fixation of 48 pounds of nitrogen per acre was noted. With sweet clover there was a fixation of 23 pounds per acre. In a second year's work on the same soils (6) 10 pounds of nitrogen per acre was fixed by alfalfa compared with 18 the first year. In the second test with the same crop 37 pounds was fixed against 48 the preceding year. Calculations based on analyses of the entire plants of alfalfa and sweet clover, from various areas of 1 square yard each, show 118 pounds of nitrogen fixed per acre by alfalfa while with sweet clover 76 pounds was fixed. When lined, the sweet clover showed a fixation of 133 pounds of nitrogen per acre.

Lipman and Blair (24) using cylinder experiments with various legumes turned under as green manures in a rotation of corn, potatoes, oats and rye, found a gain of 54 pounds of nitrogen annually over a period of 7 years.

Later experiments by the same authors (25) with soybeans grown in pots on a poor sandy soil and on a good silt loam, showed increases up to 624 mgm. and 208 mgm. of nitrogen, respectively, with various commercial cultures and with soil used for inoculation. On the poor soil the average fixation with all inoculants was 333 mgm., or 139 pounds per acre, while with the richer soil only 78 mgm., or 32 pounds per acre, was fixed on the average.

Pot tests by Fred and Graul (16) at Wisconsin, showed a gain of 20 pounds of nitrogen per acre from inoculated alfalfa and 59 pounds per acre when lime was applied. With soybeans in similar pot tests there was a fixation of 33 pounds of nitrogen per acre resulting from inoculation and of 64 pounds per acre when lime was applied. Later experiments with alfalfa on an acid Colby silt loam showed a fixation of 140 pounds of nitrogen per acre from inoculation and 245 pounds per acre when lime was applied. When the nitrogen of the roots was determined also the fixation became 164 pounds and 264 pounds respectively. On Sparta sand, the increases from inoculation were 81 pounds and 269 pounds per acre with and without lime for the tops alone, and for tops and roots 105 and 339 pounds per acre. With red clover on the Colby silt loam the fixation by inoculation was 5.9 pounds and 62 pounds per acre with and without lime, while on the Sparta sand the figures were 54 pounds and 145 pounds of nitrogen fixed. The nitrogen present in the soils used in these various pot tests was determined at the beginning and at the end of the experiment, and from a calculation of the nitrogen in the seeds and in the crops removed, the actual gain in nitrogen in the experiment was calculated. With alfalfa the amounts fixed from inoculation were 206 pounds and 70 pounds per acre without lime, and 180 pounds and 323 pounds with lime. With clover the amounts were 5 pounds and 160 pounds per acre without lime, and a loss of 15 pounds and a fixation of 214 pounds with lime.

Blair (9) studied the effect of both cowpeas and soybeans as green manures on the yields of wheat and rye in pot tests and found an average gain of 15 pounds of nitrogen annually for the two crops, for the last 4 years of an 8-year period. The 8-year average amounted to about 12 pounds.

Brown (10) found that the inoculation of alfalfa by various cultures and by soil brought about an average fixation of 17 pounds of nitrogen per acre and 14 pounds per acre in two series of plot tests. With cowpeas, the fixation amounted to 28 pounds and with soybeans to 41 pounds of nitrogen per acre.

Experiments in pots by Fellers (15) showed an increase of 1 to 3 per cent in the protein content of the seed of soybeans with various inoculants. In field tests, increases up to 9.5 per cent of protein in the seeds were noted.

Fred and Graul (18) in pot tests with soybeans on Hancock sand found with one crop an average increase of 100 pounds of nitrogen per acre from inoculation. Determinations of the source of nitrogen in the soybeans, by analysis of soil, seed and crop showed, for the three crops grown, that 323 pounds of nitrogen per acre was taken from the atmosphere by inoculated plants while when lime was used, 389 pounds per acre was secured. In a field test an average increase of 24 pounds of nitrogen per acre was found from inoculation.

Albrecht (2) found in the soil tested after growing one crop of soybeans and two of cowpeas, an increase in nitrogen of 107 pounds per acre. Where clover tops were added to the soil the fixation was smaller. After the first crop was grown there was a small decrease in nitrogen, but following the second crop a considerable increase occurred. It should be noted that the decrease was much smaller than the error in the determination of nitrogen while the increases were much beyond it.

It is apparent from the experiments cited that the amount of nitrogen fixed by legumes, as measured by the increased nitrogen in inoculated over uninoculated crops, is extremely variable and depends upon many soil and crop conditions. Little information is supplied, however, which shows definitely the proportion of nitrogen in inoculated legumes which is taken from the air and the proportion from the soil. Only in those tests where the nitrogen content of the soil is determined before and after the growth of the legumes is there really definite data on this point. Again the composition of the soil and other factors undoubtedly influence the results secured.

Considerable work has been carried out in studying the relative nitrogen content of the tops and roots of legumes, and also the relation between the weights of the tops and the roots. A brief summary of previous investigations along this line will be given here.

Lupton (26) in experiments with peas found, as an average of four tests, 8 per cent of the nitrogen in the roots and stubble and 92 per cent in the tops. Tests in New Jersey reported by Voorhees and Street (40) show 6 per cent of the nitrogen of cowpeas in the roots and 94 per cent in the vines. Experiments with crimson clover the succeeding year (41) show 28 per cent of the nitrogen in the roots on April 24, 8 per cent on May 12, 6 per cent on May 24 and 5 per cent on May 31.

Snyder (37) found 20 per cent of the nitrogen in clover in the roots. Waters (43) reported 32 per cent of the nitrogen in clover hay in the roots and stubble

while with crimson clover 20 per cent was in the roots and stubble. Roberts and Clinton (33) found 19 per cent of the nitrogen of crimson clover in the roots, 39 per cent with red clover and 54 per cent with mammoth clover. Duggar (11) found 16 per cent of the nitrogen of crimson clover in the roots and stubble while with hairy vetch, 18 per cent was in the roots. In a later experiment (12) with velvet beans, 6 per cent of the nitrogen was in the roots and stubble and 94 per cent in hay. In a still later test by the same investigator (13) with hairy vetch, 14 per cent of the nitrogen was in the roots and stubble on April 19, just before blooming, 13 per cent on April 26 when 5 per cent of the blooms were showing, 11 per cent on May 2 at full bloom, and 14 per cent on May 14 when seed pods were formed but not filled.

Woods (46) determined the nitrogen in the tops and roots of various legumes and found the proportion of nitrogen in the roots of horse beans to be 16 per cent, soybeans 5 per cent and 18 per cent, cowpeas 10 per cent, vetch 15 per cent, white lupines 10 per cent, yellow lupines 10 per cent, blue lupines 9 per cent and red clover 24 per cent.

Duggar (14) testing the use of cowpeas as a fertilizer found that at the blooming stage 7 per cent of the nitrogen was in the roots and stubble, 78 per cent in the vines and 15 per cent in the fallen leaves and leaf stalks. At the ripening stage the corresponding figures were 9 per cent, 65 per cent and 26 per cent. Penny (29) grew crimson clover on a sandy soil and on a clay soil, and on April 22, he found 31 per cent of the nitrogen in the roots on the sandy soil and 22 per cent on the clay soil. On May 22 the corresponding figures were 29 per cent and 11 per cent. He suggests that the difference in the case of the two soils is partly due to the greater ease of recovering the roots on the sandy soil and to the fact that the tops of the plants increased less on the sandy soil in the interval of sampling. In later tests with various legumes as cover crops the same investigator (30) found with soybeans 6 per cent of the nitrogen in the roots, with cowpeas 6 per cent, with vetches, 10 per cent, with crimson clover 5 per cent, with alfalfa 42 per cent, and with red clover 32 per cent.

Hopkins (21) in experiments with cowpeas found that average results from three different lots of 10 plants each, showed that 86 per cent of the nitrogen in inoculated plants was in the tops, 5 per cent in the roots and 9 per cent in the tubercles, while with the uninoculated plant, 7 per cent was in the roots and 93 per cent in the tops. In tests with red clover the same author (22) reports 25 per cent of the total plant nitrogen in the surface roots (0-7 in.) while only 1 per cent was in the lower roots. With cowpeas, 12 per cent was in the subsurface roots and one per cent below and with soybeans, the corresponding amounts were 8 per cent and 1 per cent. With sweet clover 14 per cent of the total nitrogen was found in the roots and 86 per cent in the tops.

Experiments by Shutt (34) showed 19 per cent of the nitrogen in poorly inoculated horse beans in the roots, and 25 per cent in better inoculated plants.

With mammoth red clover, 40 per cent of the nitrogen was in roots. In a field test, mammoth red clover seeded with barley, showed the subsequent year 28 per cent of the nitrogen in the roots.

The most complete study of any legume was made of crimson clover by Penny (31). He found that the proportion of nitrogen content of the roots to that of the whole plant ranged from 12 to 50 per cent and averaged about 30 per cent. The proportion fluctuated greatly and had little connection with the stage of growth. The yield of nitrogen 30 days before full bloom when the crop was on the average just half grown, ranged from one-half to four-fifteenths of the yield at full bloom. On soil accustomed to the crop little nitrogen was gained during the last month, while on new soil much was taken up late in growth. In one experiment the proportion of nitrogen in the roots 32 days before full bloom was 19 per cent, 27 days beore full bloom 20 per cent, 18 days before full bloom 1.9 per cent, 8 days before full bloom 25 per cent, at full bloom 26 per cent and 30 days after full bloom, 20 per cent.

In a second test 29 days before full bloom, 34 per cent was in the roots, 20 days before full bloom 34 per cent, 8 days before full bloom 36 per cent and at full bloom, 43 per cent. In a third test 24 days before full bloom, 22 per cent was in the roots, and at full bloom, 25 per cent. In a fourth test the figures for 25 days before and at full bloom were 32 per cent and 39 per cent, respectively. In a fifth test, 26 days before full bloom, 22 per cent was in the roots while at full bloom only 11 per cent was found. In every case the total nitrogen present in the entire plant increased up to full bloom and then decreased. On the average 25 per cent of the total nitrogen of the crop was underground. When the stubble and roots were plowed under it was assumed that 35 to 40 per cent of the nitrogen was added to the soil. No information is given as to the amount of "new" nitrogen taken up by the plant, although it is suggested that the amount of "new" nitrogen is probably smaller on a soil rich in that element.

Smith and Robison (36) in three tests found 4 per cent, 6 per cent, and 6 per cent of the nitrogen in inoculated soybeans in the roots, while with inoculated cowpeas 9 per cent of the nitrogen was in the roots. Woll and Olson (45) found 4 per cent of the nitrogen in inoculated soybeans in the roots, while in uninoculated plants 1 per cent was in the roots. Penny and MacDonald (32) in experiments with crimson clover found 19 per cent of the nitrogen in the roots in the first fall growth. The next year at the middle stage of growth there was 31 per cent in the roots and at full bloom, 25 per cent. Alway and Pinckney (4) found 31 per cent of the nitrogen of alfalfa plants in the roots on August 3, the first year, while on June 4 of the next year 17 per cent was in the roots. Wiancko, Fisher and Cromer (44) found 11 per cent of the nitrogen in soybeans and 14 per cent of that in cowpeas, in the roots.

Arny and Thatcher (5) experimenting with alfalfa showed 25 per cent of the nitrogen on the average in the roots, with sweet clover 12 per cent was in

the roots. In later experiments by the same investigators (6) with inoculated alfalfa, 21 per cent of the nitrogen was in the roots, but with sweet clover they found only 1.5 per cent in the roots.

Fred and Graul (16) found 31 per cent of the nitrogen in inoculated alfalfa in the roots, while when lime was applied to the soil, 30 per cent was in the roots. On another soil 15 per cent was in the roots when unlimed, while with lime added 10 per cent was found.

Albrecht (2) found with the first crop of cowpeas on an untreated soil, 27 per cent of the nitrogen in the roots. When nitrates were added to the soil the percentage was slightly increased and with larger amounts slightly reduced. In the second crop on the same soil, 15 per cent of the nitrogen was in the roots. Little effect was noted from the addition of nitrates, the smallest application giving a slight increase in the per cent of nitrogen in the roots. On the second soil which had grown soybeans, 21 per cent of the nitrogen was in the roots in the first crop and 17 per cent in the second. With additions of clover tops to the soil, the per cent in the roots was reduced gradually in the first crop, the largest amount showing the greatest reduction. With the second crop a reduction occurred but it was not consistent and the largest application had the smallest effect.

It is apparent from the data given that the actual proportions of nitrogen in the tops and roots of legumes is extremely variable and depends upon many conditions. Hopkins (22) concludes that on the average one-third of the nitrogen of red clover is in the roots. With alfalfa he believes that a larger amount is in the roots, possibly one-half as much is in the roots as is removed by the crop even when the plants are several years old. With cowpeas and soybeans he suggests that as a rule not more than one-tenth of the nitrogen is in the roots and stubble. These estimates may be fairly accurate but at best they are only estimates and variations of considerable moment may frequently occur. Accurate information along this line can be secured only by many experiments on a wide variety of soils under a broad range of crop conditions. The work reported in the following pages was planned to throw some light on this interesting and important problem by securing analytical data both on legumes and on the soils on which they were grown.

EXPERIMENTAL

Two soils were used in this experiment, both glacial in origin, occurring in the Wisconsin drift soil area. One is classified as the Carrington loam by the Bureau of Soils and the other as Miami fine sandy loam. These soils were chosen because of their different characteristics particularly as regards organic matter content. The former is dark brown to black in color and well supplied with humus and nitrogen while the latter is light in color and much lower in organic matter and nitrogen. Analyses showed 0.2701 per cent of nitrogen in the Carrington loam and 0.1353 per cent in the Miami fine sandy loam.

In the various experiments, clover and alfalfa were grown on these two soils, in some cases without treatment, that is, unsterilized and uninoculated, and in the others sterilized and inoculated with cultures containing the proper bacteria to bring about inoculation. The sterilization was accomplished by autoclaving for 1 hour.

Large samples of the two soils were secured from the field, sieved and either filled into pots directly or sterilized and then weighed in pots. Eight pots were filled with about 10 pounds of soil in each test, enough soil being employed so that the pots were well filled, and the exact weight of dry soil was determined in each case. Clover or alfalfa was seeded in the various groups of pots, pure cultures added to the sterilized series and the moisture content was brought up to the optimum by adding water to weight. The moisture content was kept up during the continuance of the experiment by weighing the pots and adding water to weight. The same number of plants were grown in each pot both with the clover and with the alfalfa.

In each test the crop was harvested from duplicate pots at various stages of growth, the times chosen being two weeks before blooming, when the blooms appeared, at full bloom and when mature. The green and dry weights of the crops were secured and the roots were carefully and completely removed from the soil, dried and weighed. The nitrogen content of both the tops and the roots was then determined by the Kjeldahl method on duplicate samples of each. The nitrogen content of the soil also was determined after the removal of the crops. Four determinations were made on each soil and the results were required to agree very accurately, repeats being run in all necessary cases so that the results agreed to the fourth decimal place. Only the final average results are given in the tables.

Series I

In this series clover was grown on the Carrington loam, unsterilized and uninoculated. The weights of crop secured, tops, roots and plants, the per cent of nitrogen in the tops and roots and the total nitrogen in tops, roots and plants are given for each pot in table 1. The averages of these results and calculations showing the per cent of plant growth in tops and roots and the per cent of total nitrogen in the tops and roots, in the duplicate tests are shown in table 2.

Examining this latter table it is evident first of all that the largest increase in crop occurred between 2 weeks before blooming and when the blooms appeared. Further increases occurred up to maturity. About 31 per cent of the total weight of the plants was found in the roots as an average of the results at the four stages of growth. The largest amount, 34 per cent, was in the roots when the blooms appeared. This was followed at the time of full bloom by a decrease to 29 per cent and at maturity 32 per cent was found in the roots. These differences, however, are not large enough to be very definite.

TABLE 1

Clover in unsterilized, uninoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	5.19		2.30		7.49		3.190		2.400		0.1656		0.0552		0.2208	
2	5.05	5.12	2.20	2.25	7.25	7.37	3.510	3.350	2.500	2.450	0.1773	0.1714	0.0550	0.0551	0.2323	02265
3	39.30		22.35		61.65		2.780		1.725		1.0925		0.3855		1.4780	
4	48.65	43.97	23.85	23.10	72.50	67.07	2.575	2.677	1.655	1.690	1.2527	1.1726	0.3947	0.3901	1.6474	1.5627
5	70.15		29.81		99.96		2.465		1.593		1.7292		0.4749		2.2041	
6	47.10	58.62	18.85	24.33	65.95	82.95	2.289	2.377	1.379	1.486	1.0781	1.4036	0.2599	0.3674	1.3380	1.7710
7	62.30		30.65		92.95		2.031		1.121		1.2653		0.3436		1.6089	
8		62.30		30.65		92.95		2.031		1.121		1.2653		0.3436		1.6089

TABLE 2

Clover in unsterilized, uninoculated Carrington loam

SAMPLING	AVERAGE WEIGHT			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	5.12	2.25	7.37	69.47	30.53	0.1714	0.0551	0.2265	75.67	24.33
When blooms appeared	43.97	23.10	67.07	65.56	34.44	1.1726	0.3901	1.5627	75.03	24.97
Full bloom	58.62	24.33	82.95	70.66	29.24	1.4036	0.3674	1.7710	79.25	20.75
Mature	62.30	30.65	92.95	67.02	32.98	1.2653	0.3436	1.6089	78.64	21.36

The total nitrogen in the tops was the greatest at full bloom and decreased somewhat at maturity. In the roots there was the largest amount of nitrogen when the blooms appeared, small decreases occurring up to maturity. In table 1, the percentage of nitrogen in the tops and roots is shown to be the greatest 2 weeks before blooming, considerable decreases occurring at each succeeding stage of growth. The largest amount of total nitrogen in the plants was found at full bloom, a decrease occurring at maturity.

The largest per cent of the total nitrogen was found in the roots when the blooms appeared, although the difference was not very great over the amount in the roots 2 weeks earlier. At full bloom a much smaller proportion was present in the roots and at maturity a small increase had occurred. On the average about 22 per cent of the total nitrogen was found in the roots.

TABLE 3

Clover in unsterilized, uninoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.2678	10.5543	0.2208	10.7751	10.6449	0.1302		
2	Two weeks before blooming	0.2678	10.5543	0.2323	10.7866	10.6449	0.1417	0.1359	1.1
3	When blooms appeared	0.2564	10.1050	1.4780	11.5830	10.6449	0.9381		
4	When blooms appeared	0.2638	11.1136	1.6474	12.7610	11.3790	1.3820	1.1600	9.6
5	Full bloom	0.2616	10.4284	2.2041	12.6325	10.7673	1.8652		
6	Full bloom	0.2671	10.6477	1.3380	11.9857	10.7673	1.2184	1.5418	12.8
7	Mature	0.2567	10.1168	1.6089	11.7257	10.6449	1.0808		
8	Mature	0.2446				12.3579		1.0808	9.0

In table 3 appear the results of the nitrogen determinations on the soils before and after cropping and the calculations of the gains in nitrogen by the growth of the legume or the nitrogen fixed from the atmosphere, figured in grams per pot. The results are calculated as centigrams per plant and not in pounds per acre as the crop yields are so much greater than are ever obtained in the field, and as a consequence the increases in nitrogen are far greater than could occur in the field. Evidently the red clover bacteria were present in the soil, for a large fixation of nitrogen occurred and the plants were thoroughly inoculated.

No account is taken in these calculations of the nitrogen fixed by *Azotobacter* in these pots and in some of the experiments, as will be noted later, there was evidently some action of these non-symbiotic organisms. The process of azofication is of such common occurrence however, that without special precautions it could not be eliminated in these tests, particularly with-

out sterilizing the soil and as the amounts fixed are small (amounting to not more than 0.100 gm. per pot in special tests on the same soils) compared with those assimilated by the legumes, the process is not generally considered. It is apparent from these results that there was an increase in the amount of nitrogen fixed by the plants up to the period of full bloom but beyond that a decrease occurred. Only one pot being harvested at maturity, however, renders the results at that time uncertain and they should not be taken as conclusive. At full bloom the amount of nitrogen fixed amounted to 12.8 cgm. per plant, tops and roots, and 1.1 cgm. per plant, roots alone. These figures indicate to what extent the growth of an inoculated crop of red clover may enrich the soil in nitrogen when it is used as a green manure, or when it is removed for hay and only the roots remain in the soil. Calculating these gains in nitrogen in per cent of the total amount in the plants, the following figures are secured:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	60.0
When blooms appeared	74.3
Full bloom	87.1
Mature	67.1

At full bloom a very large proportion of the total nitrogen of the plants was taken from the air. Other results may show a still further increase at maturity or at least no decrease, and the decrease noted here was probably due to some unusual condition in the one pot which was carried to maturity.

It is interesting also to calculate the proportion of the nitrogen present in the tops only of the clover, which was fixed from the atmosphere. Utilizing the figures from table 2, which show the total nitrogen in the tops and roots, the following figures are secured:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	79.2
When blooms appeared	98.9
Full bloom	100.0*
Mature	85.4

* Some of the nitrogen in the roots came from the air in this case and the actual figure here is greater than 100 per cent.

The proportion of nitrogen in the tops taken from the air increased up to full bloom and then decreased. In fact, at full bloom, all the nitrogen of the tops was taken from the atmosphere and a part of that in the roots also came from the air. The results secured at maturity were not definite and

hence conclusions are difficult, but the results of the series as a whole indicate that with inoculated red clover on this soil not only may all the nitrogen in the tops represent "new" nitrogen taken from the air, but the roots may also contain more than "old" nitrogen secured from the soil.

Series II

In this series clover was grown on Carrington loam, sterilized and inoculated. The weights in grams of the tops and roots of the plants, the percentage of nitrogen in the tops and roots and the total nitrogen in the tops, roots and plants are given in table 4. The average results from these figures, the percentage of the total plant growth in the tops and roots and the percentage of the total nitrogen of the plant in the tops and roots are shown in table 5.

It is evident from the figures in this table, first of all that the total weight of the plants increased up to maturity, the largest increase occurring between the stages of 2 weeks before blooming and when the blooms appeared. A large increase also was found from the latter period to the period of full bloom. In the case of the tops, the increases were much the same proportionately as of the entire plant but with the roots the increases were small after the first period. The largest percentage of plant growth in the roots was found when the blooms appeared, smaller proportions being present at the later stages of growth. This is in accord with the results obtained in the preceding series. The average proportion of the total plant growth in the roots at the four stages of growth was 30 per cent. This figure is slightly smaller than that secured in the previous series but checks very well with it.

The total nitrogen of the plants increased up to maturity but the greatest increases occurred between the first and second periods just as was noted in the case of the weights of the crop. In table 4 it is seen that the greatest percentage of nitrogen was found in both tops and roots at the first stage of growth. At subsequent periods the proportion decreased, the differences, however, being small. At the last two stages the greatest decrease occurred between the first period and the second. The largest percentage of the total nitrogen of the plants in the roots is shown in table 5 to be present at the first stage of growth, the amounts decreasing at the later periods, only 19.7 per cent being found in the roots at maturiy. The average proportion of the total nitrogen in the roots at all stages of growth was about 22 per cent, exactly the same figure as was obtained in the preceding series. The results thus far are therefore apparently very much the same on the Carrington loam whether unsterilized or sterilized and inoculated. The inoculating bacteria were evidently present in the soil and whether or not they were as effective as those introduced into the sterile soil cannot be determined on account of the sterilization. The sterilization of the soil, however, apparently did not cause any interference with the best growth of the crop, an effect which is frequently noted when soil is subjected to steam under pressure.

TABLE 4

Clover in sterilized, inoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	gm.	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	5.98		2.48		8.46		3.270		2.670		0.1955		0.0662		0.2617	
2	5.54	5.76	2.57	2.52	8.11	8.28	3.440	3.355	2.720	2.695	0.1905	0.1930	0.0699	0.0680	0.2604	0.2610
3	46.85		22.80		69.65		2.790		1.860		1.3071		0.4240		1.7311	
4	55.40	51.13	30.99	26.89	86.39	78.02	2.550	2.670	1.550	1.705	1.4127	1.3599	0.4803	0.4521	1.8930	1.8120
5	89.45		31.70		121.15		2.440		1.582		2.1826		0.5015		2.6841	
6	74.10	81.77	36.72	34.21	110.82	115.98	2.457	2.448	1.600	1.591	1.8206	2.0016	0.5875	0.5445	2.4081	2.5461
7	89.00		38.93		127.93		2.291		1.324		2.0389		0.5154		2.5543	
8	97.10	93.05	29.95	34.44	127.05	127.49	2.365	2.328	1.829	1.576	2.2964	2.1676	0.5478	0.5316	2.8442	2.6992

TABLE 5

Clover in sterilized, inoculated Carrington loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	5.76	2.52	8.28	69.56	30.44	0.1930	0.0680	0.2610	73.94	26.06
When blooms appeared	51.13	26.89	78.02	65.53	34.47	1.3599	0.4521	1.8120	75.05	24.95
Full bloom	81.77	34.21	115.98	70.58	29.42	2.0016	0.5445	2.5461	78.61	21.39
Mature	93.05	34.44	127.49	72.98	27.02	2.1676	0.5316	2.6992	80.30	19.70

In table 6 appear the results of the nitrogen determinations on the soil before and after growth of the legume and the calculations showing the nitrogen fixed by the crop. The gain in nitrogen is calculated in grams per pot and as centigrams per plant. Examining this table there is found to be a gradually increasing fixation of nitrogen from the atmosphere at succeeding stages of growth. Thus at full bloom twice as much nitrogen was fixed as at the preceding stage, while at maturity a further increase is noted. The greatest gain apparently occurred between the second and third stages of growth. In the preceding series the greatest gain took place between the first and second stages. This difference may have been due to differences in the growth of the plants or to variations in the rate of inoculation. It is interesting to note

TABLE 6

Clover in sterilized, inoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGD GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.2704	11.0242	0.2617	11.2859	11.0120	0.2739		
2	Two weeks before blooming	0.2685	10.9467	0.2604	11.2071	11.0120	0.1951	0.2345	1.9
3	When blooms appeared.	0.2540	10.0104	1.7311	11.7415	10.6449	1.0966		
4	When blooms appeared.	0.2468	11.7390	1.8930	13.6320	12.8473	0.7847	0.9406	7.8
5	Full bloom	0.2543	10.8286	2.6841	13.5127	11.5014	2.0113		
6	Full bloom	0.2524	10.6333	2.4081	13.0414	11.3790	1.6624	1.8368	15.3
7	Mature	0.2566	10.4616	2.5543	13.0159	11.0120	2.0039		
8	Mature	0.2519	10.3811	2.8442	13.2253	11.1343	2.0910	2.0474	17.0

that in this series the fixation increased up to maturity, thus indicating that in the preceding test, as was noted there, the results at maturity were probably abnormal. The actual amount of nitrogen fixed was greater on the sterilized inoculated soil in all but one case, which was at the second stage of growth. This may be taken to indicate that the organisms introduced into the sterilized soil proved more effective than those present in the unsterilized soil. The differences are not great, however, especially at the full-bloom stage, but they are great enough to be important if the value of the nitrogen fixed were figured on the basis of the market price of the element in commercial fertilizers.

The percentage of the total nitrogen in the plants taken from the air is shown in the following figures:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	89.8
When blooms appeared	51.9
Full bloom	72.1
Mature	75.8

The largest percentage fixation was found at the first stage. Following that period there was a decrease in the proportion fixed but at the later stages the percentages increased considerably. At maturity three-fourths of the nitrogen of the plants apparently came from the air. This amount is somewhat greater than that noted in the preceding test but the percentage fixed at full bloom is smaller than that in the other test and the same is true at the second stage of growth. These results probably indicate something of the differences which occur in the field when legumes are inoculated in different ways and may be a reflection of the rapidity or completeness with which the plants become inoculated. It is also quite possible that the sterilization exerted some influence on the rate of inoculation or on the rate at which nitrogen from the soil was supplied to the plants. At the later stages of growth, however, the greater efficiency of the organisms probably brought about the larger fixation and also the larger crop growth.

The percentage of the nitrogen in the tops of the crop, which came from the atmosphere is shown in the following figures:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	100.0*
When blooms appeared	69.1
Full bloom	91.7
Mature	94.4

* Over 100 per cent.

At the first stage all the nitrogen in the tops was taken from the air and a part of that in the roots was secured from the air also. At the second stage only 69 per cent of the total amount in the tops came from the air. At the later samplings, however, almost all of the nitrogen was taken from the air. Again these variations may be due to the differences in rate of inoculation, rate of growth or efficiency of the organisms but it is evident that practically all of the nitrogen in the tops of the clover came from the atmosphere and the amount present in the roots evidently was taken from the soil. Small differences cannot be considered of great significance in this work, since such small amounts of nitrogen and such small variations in percentage are involved.

Series III

In series III alfalfa, was grown on Carrington loam unsterilized and uninoculated. Table 7 shows the weights of the tops and roots at the different stages of growth, the percentage of nitrogen in the tops and roots and the total nitrogen present in the tops, the roots and the whole plants. The averages of these figures are given in table 8 and calculations also are given showing the per cent of total plant growth present in the tops and roots and the per cent of the total nitrogen of the crop in the tops and roots. As was noted with the clover the crop increased in weight up to maturity, the greatest increase occurring between the first and second periods. A large gain also was found between the second and third periods, but from full bloom to maturity only a relatively small increase occurred. Very much the same increases as these are noted in the case of both the tops and the roots, and the increases at the different stages were quite similar with the two portions of the plants. Apparently with this crop the tops and roots develop at about the same rate. The only difference noted is at the second stage when the roots showed a greater increase than did the tops. The greatest percentage of the total weight of the plants was found in the roots at the second stage of growth while at later stages the figures were somewhat smaller. At the first stage only a small proportion of the plant was in the roots but the roots soon began to develop and made a very rapid growth before the appearance of blooms. On the average over 41 per cent of the plant was in the roots, and if the figures at the first stage are not included the average shows over 50 per cent of the crop present in the roots. These figures indicate that alfalfa has a larger proportion of roots to tops than does red clover.

From table 7 it will be seen that the greatest percentages of nitrogen were present both in the tops and roots at the first stage of growth. At later stages decreases occurred. In the case of the tops these decreases continued up to maturity but with the roots a slight increase occurred at the third period. This, however, was followed by a decrease at maturity. The total nitrogen in the plants increased up to full bloom and decreased slightly at maturity. The difference here was not great and probably should not be taken as conclusive. The largest percentage of the total nitrogen in the roots was found at maturity but the differences were not great after the second stage of growth. The largest gain of nitrogen in the roots occurred between the first and second stages. On the average over 35 per cent of the total nitrogen of the alfalfa was in the roots and if the figures at the first period are not included, 43 per cent is found in the roots. This is about twice as much as was found in the case of the clover in the two preceding series.

In table 9 are given the results of the nitrogen determinations and the calculations of the gain in nitrogen in the pots and per plant, or the nitrogen fixed by the alfalfa. Evidently the proper bacteria were present in the soil, for the crop became inoculated and a large fixation of nitrogen occurred.

TABLE 7

Alfalfa in unsterilized, uninoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	3.35		0.49		3.84		3.540		3.180		0.1186		0.0156		0.1342	
2	3.14	3.24	0.60	0.54	3.74	3.78	3.370	3.455	3.040	3.110	0.1058	0.1122	0.0182	0.0169	0.1240	0.1291
3	21.05		27.75		48.80		3.097		1.834		0.6519		0.5089		1.1608	
4	22.25	21.65	22.95	25.35	45.20	47.00	3.050	3.073	1.917	1.875	0.6786	0.6652	0.4400	0.4745	1.1186	1.1397
5	33.70		37.20		70.90		2.803		2.290		0.9446		0.8519		1.7965	
6	53.90	43.80	47.50	42.35	101.40	86.15	2.426	2.614	2.018	2.154	1.3076	1.1261	0.9586	0.9052	2.2662	2.0313
7	48.10		48.00		96.10		2.471		1.780		1.1886		0.8544		2.0430	
8	46.00	47.05	46.40	47.20	92.40	94.25	2.036	2.253	1.897	1.838	0.9366	1.0626	0.8802	0.8673	1.8168	1.9299

TABLE 8

Alfalfa in unsterilized, uninoculated Carrington loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL OF NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	3.24	0.54	3.78	85.71	14.29	0.1122	0.0169	0.1291	86.91	13.09
When blooms appeared	21.65	25.35	47.00	46.06	53.94	0.6652	0.4745	1.1397	58.36	41.64
Full bloom	43.80	42.35	86.15	50.84	49.16	1.1261	0.9052	2.0313	55.43	44.57
Mature	47.05	47.20	94.25	49.92	50.08	1.0626	0.8673	1.9299	55.06	44.94

TABLE 9

Alfalfa in unsterilized, uninoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.2696	10.8695	0.1342	11.0037	10.8896	0.1141		
2	Two weeks before blooming	0.2699	12.3487	0.1240	12.4727	12.3579	0.1148	0.1144	1.2
3	When blooms appeared	0.2572	10.4860	1.1608	11.6468	11.0120	0.6348		
4	When blooms appeared	0.2555	7.8707	1.1186	8.9893	8.3199	0.6694	0.6521	7.2
5	Full bloom	0.2528	11.2228	1.7965	13.0193	11.9908	2.0285		
6	Full bloom	0.2548	10.3728	2.2662	12.6490	10.8896	1.7594	1.8938	21.0
7	Mature	0.2789	11.2444	2.0430	13.2874	10.8896	2.3978		
8	Mature	0.2778	11.4517	1.8168	13.2685	11.1343	2.0942	2.2460	24.9

An increase in nitrogen fixed was noted at each successive stage of growth, at maturity 24.9 cgm. of nitrogen per plant being obtained from the atmosphere. The greatest increase occurred between the second and third stages of growth. With the roots alone the fixation amounted to 12.4 cgm. per plant.

The percentage of the total nitrogen in the plants taken from the atmosphere is shown in the following figures:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	88.6
When blooms appeared	57.2
Full bloom	93.2
Mature	100.0*

* Over 100 per cent.

The largest percentage of total nitrogen taken from the air probably was in the plants at maturity. The figures, however, are uncertain, inasmuch as there was evidently some fixation of nitrogen by non-symbiotic bacteria. The soil showed a gain in nitrogen apart from that fixed by the legumes and that gain probably came from the action of the azofiers. At full bloom, however, almost all of the nitrogen of the plants (tops and roots) came from the atmosphere. The smallest proportion of nitrogen from the air was taken by the plants at the second stage of growth, while at the first stage a large amount came from the atmosphere.

Calculating the percentage of the nitrogen in the tops taken from the air, the following figures are obtained:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	100.0*
When blooms appeared	98.0
Full bloom	100.0*
Mature	

* Over 100 per cent.

At every stage of growth practically the entire amount of nitrogen in the tops was fixed from the air and at the last two stages the amount in the roots also was very largely secured from the atmosphere. The interference of the non-symbiotic organisms makes the conclusions difficult but at least it may be said that the entire amount in the tops was taken from the air and probably a portion of that in the roots was secured from the same source.

Series IV

In this series alfalfa was grown on Carrington loam sterilized and then inoculated. The weights of the tops and roots and the nitrogen present in each are shown in table 10. The averages from the duplicate pots and the calculations of the percentages of plant growth in the tops and roots and of the proportion of the total nitrogen in the tops and roots are given in table 11.

From the figures given in the latter table it is apparent that the weight of the plants increased at each stage of growth, the largest increase occurring at the third stage. The gain from the first to the second stage, however, was almost as great. This is in accord with the results secured on the unsterilized soil. As in that case also the tops and roots increased in a very similar way, the largest increase in both cases occurring between the second and third stages. The increase in both tops and roots was small from full bloom to maturity. In this series the gain at the second stage in the roots was not as great as that in the tops, differing from the results of the preceding test. This difference may be due to the difference in the soil conditions brought about by the sterilization, possibly a bacterial variation, but the figures are not sufficiently far apart to warrant conclusions.

The largest percentage of the total nitrogen of the plants was found in the roots at maturity just as in the preceding case. The greatest increase also, as noted before, occurred from the first to the second stages of growth. A slightly smaller percentage was in the roots at maturity, but the difference was not great. The average percentage in the roots at all stages was 34, as against 35 per cent in the preceding test, and if the figures obtained 2 weeks before blooming are not included the average is 39 per cent against 43 per cent. These differences again may be due to the soil conditions. From table 10 it appears that the percentage of nitrogen in the tops decreased at each

TABLE 10

Alfalfa in sterilized, inoculated Carrington loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	2.10		0.57		2.67		3.710		3.600		0.0779		0.0205		0.0984	
2	3.72	2.91	0.45	0.51	4.17	3.42	3.130	3.420	3.770	3.685	0.1164	0.0972	0.0170	0.0187	0.1334	0.1159
3	27.32		19.55		46.87		2.190		1.720		0.5983		0.3363		0.9346	
4	23.72	25.52	22.15	20.85	45.87	46.37	2.900	2.545	1.750	1.735	0.6879	0.6431	0.3876	0.3619	1.0755	1.0050
5	52.10		46.80		98.90		2.775		1.927		1.4457		0.9018		2.3475	
6	46.00	49.05	39.21	43.00	85.21	92.05	2.191	2.483	1.907	1.917	1.0078	1.2267	0.7477	0.8248	1.7555	2.0515
7	54.75		52.03		106.78		2.129		1.870		1.1656		0.9730		2.1386	
8	54.20	54.47	50.75	51.39	104.95	105.86	2.517	2.323	1.774	1.822	1.3642	1.2649	0.9003	0.9366	2.2645	2.2015

TABLE 11

Alfalfa in sterilized, inoculated Carrington loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	2.91	0.51	3.42	85.09	14.91	0.0972	0.0187	0.1159	83.86	16.14
When blooms appeared	25.52	20.85	46.37	55.04	44.96	0.6431	0.3619	1.0050	63.99	36.01
Full bloom	49.05	43.00	92.05	53.28	46.72	1.2267	0.8248	2.0515	59.79	40.21
Mature	54.47	51.39	105.86	51.45	48.55	1.2649	0.9366	2.2015	57.45	42.55

stage of growth, the greatest decrease occurring at the second stage. With the roots the results vary somewhat, a large decrease occurring at the second stage, this being followed by an increase at the third period and a slight decrease at maturity. These results agree exactly with those secured in the preceding series.

In table 12 appear the results of the nitrogen determinations on the soil and the calculations of the nitrogen fixed by the crop.

TABLE 12

Alfalfa in sterilized inoculated Carrington loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.2678	10.9182	0.0984	11.0166	11.0120	0.0046		
2	Two weeks before blooming	0.2730	10.7592	0.1334	10.8926	10.6449	0.2477	0.1261	1.4
3	When blooms appeared	0.2595	11.6378	0.9346	12.5724	12.1132	0.4592		
4	When booms appeared	0.2542	11.0546	1.0755	12.1301	11.7461	0.3840	0.4216	4.6
5	Full bloom	0.2548	11.1962	2.3475	13.5437	11.8685	1.6752		
6	Full bloom	0.2602	10.3726	1.7555	12.1281	10.7673	1.3608	1.5180	16.8
7	Mature	0.2582	10.4098	2.1386	12.5484	10.8896	1.6588		
8	Mature	0.2637	10.3927	2.2645	12.6572	10.6449	2.0123	1.8355	20.3

The fixation of nitrogen here was somewhat less than that found in the unsterilized Carrington loam. Increases in fixation were noted up to maturity, the greatest increase occurring at the third stage of growth. Inoculation was thorough in this case and the nitrogen fixed shows quite accurately the ability of the legume to utilize the nitrogen of the atmosphere.

Comparisons of the fixation on this soil sterilized and inoculated with that occurring when the soil was not sterilized are not possible, on account of the possibility of somewhat greater action of the azofiers in the latter case but it would seem that the amount of nitrogen fixed by the legumes was about the same in the two cases.

Calculations of the percentage of the total nitrogen in the plants taken from the air are shown below:

SAMPLING	NITROGEN IN THE PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	41.9
Full bloom	73.9
Mature	83.3

Two weeks before blooming the entire nitrogen supply seems to have been taken from the air, but as the results are not satisfactory, the duplicate pots not agreeing, conclusions should not be drawn. At the later stages increasingly large proportions of the nitrogen came from the air until at maturity 83.3 per cent was secured in this way. The largest increase occurred between the second and third stages. Comparisons with the results on the unsterilized soil show that a somewhat smaller percentage of the total nitrogen in the plants came from the air, which may be due to a fixation of nitrogen by the azofiers in the unsterilized soil.

The percentage of the nitrogen in the tops taken from the atmosphere is shown in the following figures:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	65.5
Full bloom	100.0*
Mature	100.0*

* Over 100 per cent.

At the first stage of growth the results are unsatisfactory, as noted earlier. At the second stage 65 per cent of the nitrogen in the tops came from the air. This compares with 100 per cent obtained in the preceding test. At the later stages all the nitrogen of the tops was taken from the atmosphere and a part of that in the roots was secured from the same source. Similar results were secured also in the test on the unsterilized soil. It seems to be evident from these results with inoculated alfalfa that all the nitrogen in the tops came from the air and in some cases a *part of that in the roots also was taken from the atmosphere.*

Series V

In this series clover was grown on unsterilized, uninoculated Miami fine sandy loam. The results in table 13 show the weights of the tops, roots and crop at the various stages of growth, the percentage of nitrogen in the tops and roots and the total nitrogen present in each. The crop was not secured on one of the pots 2 weeks before blooming and therefore the results have no check at that period. The average weights and the average nitrogen content of the tops, roots and plants are given in table 14, and the calculations of the percentage of plant growth in the tops and roots and of the percentage of total nitrogen in the tops and roots are given in the same table.

The results in this table show first of all large increases in crop growth at each succeeding stage. The largest increase occurred between the first and second periods. This was true also for the tops and roots. There was a larger gain in the case of the tops than with the roots at the second period,

TABLE 13

Clover in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1																
2	1.65	1.65	0.60	0.60	2.25	2.25	3.23	3.23	3.02	3.02	0.0533	0.0533	0.0181	0.0181	0.0714	0.0714
3	39.95		27.55		67.50		2.55		1.60		1.0187		0.4408		1.4595	
4	35.95	37.95	21.12	24.33	57.07	62.28	2.31	2.43	1.91	1.75	0.8304	0.9245	0.4034	0.4221	1.2338	1.3466
5	44.95		28.45		73.40		2.45		1.68		1.1012		0.4779		1.5791	
6	52.55	48.75	42.95	35.70	95.50	84.45	2.25	2.35	1.50	1.59	1.1823	1.1417	0.6442	0.5611	1.8265	1.7028
7	63.10		39.35		102.45		2.12		1.61		1.3377		0.6335		1.9712	
8	59.20	61.15	45.35	42.35	104.55	103.50	2.17	2.14	1.67	1.64	1.2846	1.3111	0.7573	0.6954	2.0419	2.0065

TABLE 14

Clover in unsterilized, uninoculated Miami fine sandy loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	1.65	0.60	2.25	73.33	26.67	0.0533	0.0181	0.0714	74.65	25.35
When blooms appeared	37.95	24.33	62.28	60.95	39.05	0.9245	0.4221	1.3466	68.65	31.35
Full bloom	48.75	35.70	84.45	57.72	42.28	1.1417	0.5611	1.7028	67.04	32.96
Mature	61.15	42.35	103.50	59.08	40.92	1.3111	0.6954	2.0065	65.34	34.66

and at the fourth period the tops increased more than did the roots. The largest percentage of the total plant growth in the roots was found at full bloom, a decrease ocurring at maturity. The smallest proportion was in the roots 2 weeks before blooming and a large increase had occurred when the blooms appeared. The average proportion of the plants in the roots was 36 per cent, which is somewhat higher than that found when clover was grown on unsterilized Carrington loam (31 per cent). This difference may be due to the varying soil conditions in these two types.

The percentage of the total nitrogen in the roots increased up to maturity, the increase being the greatest at the second period. The average percentage was 31 as against 22 per cent in the case of the Carrington loam. The differences in the soils used evidently influenced the relative development of tops and roots, and also their nitrogen content. The percentage of nitrogen in the tops

TABLE 15

Clover in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POT	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming								
2	Two weeks before blooming	0.1368	5.8872	0.0714	5.9586	5.8226	0.1360	0.1360	1.1
3	When blooms appeared	0.1340	5.3418	1.4595	6.8013	5.3936	1.4077		
4	When blooms appeared	0.1346	5.4876	1.2338	6.7214	5.5162	1.2052	1.3064	10.8
5	Full bloom	0.1322	5.7689	1.5791	7.3480	6.0065	1.3415		
6	Full bloom	0.1304	5.7299	1.8265	7.5564	5.9452	1.6112	1.4763	12.3
7	Mature	0.1318	5.7842	1.9712	7.7554	5.9452	1.8102		
8	Mature	0.1345	4.7524	2.0419	6.7943	4.7807	2.0136	1.9119	15.9

and roots as shown in table 13 decreased at each stage of growth, the largest decrease occurring at the second period. The variations at the later samplings were not very large nor definite.

In table 15 are given the results of the nitrogen determinations on the soils before and after growth of the legumes, and the gain in nitrogen expressed as grams per pot and as pounds per acre.

It is evident that the clover became thoroughly inoculated on this soil and a large fixation of nitrogen from the atmosphere took place. The largest fixation occurred at maturity, increases occurring at each period, the largest gains being from the first to the second and from the third to the fourth periods. At maturity 15.9 cgm. of nitrogen were fixed per plant, tops and roots, and 5.0 cgm. per plant, roots alone. These amounts are much greater than those secured on the unsterilized Carrington loam.

The percentages of the total plant nitrogen taken from the air are given below:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	97.0
Full bloom	86.6
Mature	95.2

The results at the first period are abnormal and were not duplicated, and hence are not included here. At the later periods the plants took most of their nitrogen from the air—the figures being very much larger than on the Carrington loam. Calculating the percentage of nitrogen in the tops taken from the atmosphere it is found that the total amount of nitrogen in the tops was secured from the air at all stages of growth and a large part of that in the roots was similarly secured. There was probably some non-symbiotic nitrogen fixation in this soil and the results are modified therefore to a small but unknown extent. Apparently, however, there was much difference in the results on this soil from those on the Carrington loam unsterilized. A much larger fixation of nitrogen occurred at maturity and a somewhat larger proportion of the nitrogen in the plants came from the air.

Series VI

In this series clover was grown on sterilized, inoculated Miami fine sandy loam. The results in table 16 show the weights of the tops and roots, the percentage of nitrogen present in each and the total nitrogen content of the tops, roots and plants. The average results and the determinations of the percentage of plant growth in the tops and roots and the percentage of total nitrogen in tops and roots are given in table 17. From the latter table it appears that the largest increase in total average weight of the plants, occurred between the first and second periods. At the later periods the increases were not so great, the smallest increase occurring at the last stage. The roots increased in weight somewhat more than the tops at the first period, but made little further gain. The tops, on the other hand, made a considerable increase at the third period. No further increase occurred at the fourth period.

The largest percentage of total plant growth in the roots was found at the second period, although there was little difference from the first. At the later periods there was a considerable decrease in the proportion of the plants in the roots. These results are somewhat different from those secured in the unsterilized, uninoculated Miami fine sandy loam in the preceding series, and it would seem that where the soil was sterilized and inoculated the roots developed much more rapidly. The difference may have been due either to the chemical difference in the soil brought about by the sterilization or to the difference in the bacterial factor or the thoroughness and rate of inoculation.

TABLE 16

Clover in sterilized, inoculated Miami fine sandy loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.64		1.25		2.89		2.930		3.720		0.0481		0.0465		0.0946	
2	0.98	1.31	1.53	1.39	2.51	2.70	3.180	3.055	2.470	3.095	0.0312	0.0396	0.0378	0.0422	0.0790	0.0818
3	21.25		17.80		39.05		2.147		1.550		0.4562		0.2759		0.7321	
4	16.25	18.75	22.70	20.25	38.95	39.00	2.211	2.179	1.170	1.360	0.3593	0.4077	0.2656	0.2708	0.6249	0.6785
5	41.00		22.10		63.10		2.078		1.440		0.8520		0.3182		1.1702	
6	27.50	34.25	20.90	21.50	48.40	55.75	2.231	2.154	1.284	1.362	0.6135	0.7327	0.2684	0.2933	0.8819	1.0260
7	34.10		13.95		48.05		2.064		1.521		0.7038		0.2122		0.9160	
8	33.90	34.00	18.44	16.19	52.34	50.19	1.888	1.976	1.568	1.544	0.6400	0.6719	0.2891	0.2506	0.9291	0.9225

TABLE 17

Clover in sterilized, inoculated Miami fine sandy loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	gm.	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	1.31	1.39	2.70	48.51	51.49	0.0396	0.0422	0.0818	48.41	51.59
When blooms appeared	18.75	20.25	39.00	48.07	51.93	0.4077	0.2708	0.6785	60.09	39.91
Full bloom	34.25	21.50	55.75	61.43	38.57	0.7327	0.2933	1.0260	71.41	28.59
Mature	34.00	16.19	50.19	67.74	32.26	0.6719	0.2506	0.9225	72.83	27.17

The average percentage of plant growth in the roots was 43 against 39 per cent on the unsterilized soil. On the Carrington loam the corresponding percentages were 30 and 31. Hence it seems evident that the percentage of total plant growth was greater in the roots on the Miami fine sandy loam whether sterilized or not. This difference must be attributed to the variations in soil conditions, and these variations may be in any one factor or in several. It is quite impossible to do more than speculate as to the cause, but it seems likely that the difference in nitrogen and organic matter may be the chief factor responsible.

The percentage of total nitrogen in the roots decreased at every period, being the greatest at the first and smallest at the last. This is exactly the reverse of the results on the unsterilized soil, where an increase was noted up

TABLE 18

Clover in sterilized, inoculated Miami fine sandy loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POTS	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.1351	5.9976	0.0946	6.0922	6.0065	0.0857		
2	Two weeks before blooming	0.1440	6.1318	0.0790	6.2108	5.6613	0.5495	0.3126	2.6
3	When blooms appeared	0.1344	6.2101	0.7321	6.9422	6.2144	0.7278		
4	When blooms appeared	0.1347	5.7358	0.6249	6.3607	5.6613	0.6994	0.7136	5.8
5	Full bloom	0.1263	5.5497	1.1702	6.7199	5.9452	0.7747		
6	Full b'oom	0.1294	5.5687	0.8819	6.4506	5.8226	0.6280	0.7013	5.9
7	Mature	0.1226	5.7204	0.9160	6.6364	6.3130	0.3234		
8	Mature	0.1269	5.1737	0.9291	6.1028	5.5162	0.5866	0.4550	3.8

to maturity. Again this difference may have been due to the changed soil conditions, chemical or bacterial, or both, in the sterilized soil. Similar results were secured on the Carrington loam both when unsterilized and when sterilized and inoculated, and hence the cause of the difference in results in the unsterilized Miami fine sandy loam must have been due to the effects of the sterilization on that soil.

From the results of this series the average percentage of total nitrogen in the roots was 36 against 31 per cent in the unsterilized soil. On the Carrington loam the average percentage was 22 in both series. Thus it would seem that on the Miami fine sandy loam, a larger percentage of the total nitrogen of the plants was in the roots—on the average. This is in accord with the total-weight results and may be due to the difference in the nitrogen and organic matter in the soils.

In table 18 appear the results of the nitrogen determinations on the soils before and after cropping, and the calculations of the nitrogen gain in centigrams per plant. At the first period there was evidently some abnormality in the soil conditions in the duplicate pots, as they do not agree closely and the average nitrogen gain is so large that it indicates the possibility of the accidental introduction of azofiers and vigorous action by them. At the second period there was a fixation of 5.9 cgm. per plant by the clover, and of 2.5 cgm. per plant for the roots alone. At the two latter stages, however, the amount of nitrogen fixed was not increased and the largest fixation is recorded at the second and third periods. This is quite different from the results on the unsterilized soil in the preceding series where the fixation increased up to the last period when 15.9 cgm. per plant was found to be taken from the air. On the Carrington loam much larger amounts were fixed and increases were noted up to maturity. The soil differences and the effect of sterilization must be held responsible for these results on the Miami fine sandy loam. The inoculation was quite complete but it may be noted that the plants were much smaller on the sterilized soil and this may have been due to some physiological effect on the crop from products formed in the sterilization. The reverse was true on the Carrington loam and in that case evidently no injurious compounds were formed in the soil when it was sterilized.

The following figures give the percentage of the total nitrogen in the plants taken from the air:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	100.0*
Full bloom	68.3
Mature	49.3

* Over 100 per cent.

The results at the first period were so abnormal that they are not given but the indications are that 100 per cent of the nitrogen was taken from the air. The same is true at the second period. At the later dates smaller percentages were found. These results are the reverse of those in the preceding series where practically all of the nitrogen came from the air. The sterilization evidently influenced the extent and efficiency of the inoculation.

In the table given below, appear the calculations of the percentage of the nitrogen in the tops taken from the atmosphere:

SAMPLING	NITROGEN IN THE TOPS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	
When blooms appeared	100.0*
Full bloom	95.7
Mature	67.7

* Over 100 per cent.

At the first two stages practically all of the nitrogen of the plants seemed to come from the air and hence at those periods the total quantity in the tops was secured from the atmosphere. At the third period about the same result was noted, but at maturity only 67 per cent of the amount of nitrogen in the tops came from the air, the remainder coming from the soil. The crop reduction at the fourth stage on this soil is accompanied by a reduction in nitrogen fixed due to decreased thoroughness or efficiency in inoculation, or perhaps to increased assimilable nitrogen production from the sterilization.

Series VII

In this series, alfalfa was grown on unsterilized, uninoculated Miami fine sandy loam. The weights of the crop (tops and roots) the percentage of nitrogen in the tops and roots and in the total crop are given in table 19. The average results and the determinations of the percentages of total plant growth in the tops and roots and of total nitrogen in the tops and roots are given in table 20.

The total weight of the alfalfa increased at each period, the greatest increase occurring at the second period. Large gains were found at the two later periods, however. The largest percentage of the total plant growth was in the roots at the last period, a large increase being noted at the second period and small increases at each succeeding period. These results are very closely in accord with those secured on the unsterilized Carrington loam except that in this case increases in the percentage of plant growth in the roots were much more regular. The total nitrogen increased in the plants up to maturity and the percentage of the total amount in the crops which was in the roots increased similarly, the largest percentage being found at maturity. This agrees very well with the results on the Carrington loam. The average figures for the soil used in this series was 38 per cent, and this compares with 35 per cent in the case of the Carrington loam. The soil differences undoubtedly influenced the proportion of the total nitrogen of the plants which occurred in the roots.

Table 21 gives the results of the nitrogen determinations and the average gains in nitrogen at the various stages of growth. Apparently the inoculation of the alfalfa was quite efficient and the amounts of nitrogen fixed were considerable. The fixation increased up to maturity, the greatest changes occur-

TABLE 19

Alfalfa in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	WEIGHT OF						NITROGEN IN				TOTAL NITROGEN IN					
	Tops	Average	Roots	Average	Plants	Average	Tops	Average	Roots	Average	Tops	Average	Roots	Average	Plants	Average
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	1.84		0.60		2.44		3.83		3.27		0.0705		0.0196		0.0901	
2	1.61	1.72	0.52	0.56	2.13	2.28	3.62	3.72	3.30	3.28	0.0583	0.0644	0.0172	0.0184	0.0755	0.0828
3	29.80		18.75		48.55		2.38		1.55		0.7092		0.2906		0.9998	
4	21.40	25.60	27.10	22.92	48.50	48.52	2.37	2.37	1.91	1.73	0.5071	0.6081	0.5176	0.4041	1.0247	1.0122
5	34.10		25.00		59.10		2.44		1.54		0.8320		0.3850		1.2170	
6	27.15	30.62	41.45	33.23	68.60	63.85	2.19	2.31	1.97	1.70	0.5945	0.7132	0.8165	0.6008	1.4110	1.3140
7	36.00		44.45		80.45		2.35		1.75		0.8460		0.7778		1.6238	
8	36.10	36.05	45.95	45.20	82.05	81.25	2.36	2.35	1.70	1.72	0.8520	0.8490	0.7811	0.7794	1.6331	1.6284

TABLE 20

Alfalfa in unsterilized, uninoculated Miami fine sandy loam

SAMPLING	AVERAGE WEIGHT OF			PLANT GROWTH IN		AVERAGE TOTAL NITROGEN IN			TOTAL NITROGEN IN	
	Tops	Roots	Plants	Tops	Roots	Tops	Roots	Plants	Tops	Roots
	gm.	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*
Two weeks before blooming	1.72	0.56	2.28	75.43	24.57	0.0644	0.0184	0.0828	77.77	22.23
When blooms appeared	25.60	22.92	48.52	52.76	47.24	0.6081	0.4041	1.0122	60.07	39.93
Full bloom	30.62	33.23	63.85	47.95	52.05	0.7132	0.6008	1.3140	54.27	45.73
Mature	36.05	45.20	81.25	44.37	55.63	0.8490	0.7794	1.6284	52.13	47.87

ring at the second and third periods. Only a comparatively small increase was noted at the fourth period. The fixation per plant was less than on the Carrington loam at the third and fourth stages, and this was probably a difference due to soil conditions.

TABLE 21

Alfalfa in unsterilized, uninoculated Miami fine sandy loam

POT NUMBER	SAMPLING	NITROGEN IN SOIL	TOTAL NITROGEN IN POT	NITROGEN IN PLANTS	NITROGEN IN SOIL AND PLANTS	NITROGEN IN POTS AT BEGINNING OF EXPERIMENT	GAIN IN NITROGEN IN POTS	AVERAGE NITROGEN FIXED PER POT	AVERAGE GAIN IN NITROGEN PER PLANT
		per cent	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*cgm.*
1	Two weeks before blooming	0.1342	5.4105	0.0901	5.5006	5.4549	0.0457		
2	Two weeks before blooming	0.1348	5.8622	0.0755	5.9377	5.8839	0.0538	0.0497	0.5
3	When blooms appeared	0.1313	5.8884	0.9998	6.8882	6.0678	0.8204		
4	When blooms appeared	0.1170	4.9417	1.0247	5.9664	5.4549	0.5115	0.6659	7.3
5	Full bloom	0.1376	5.4853	1.2170	6.7023	5.3936	1.3087		
6	Full bloom	0.1314	5.2381	1.4110	6.6491	5.3936	1.2555	1.2821	14.2
7	Mature	0.1319	5.6763	1.6238	7.3001	5.8226	1.4775		
8	Mature	0.1343	5.7796	1.6331	7.4127	5.8226	1.5901	1.5338	17.0

In the following table results are given showing the percentage of the total nitrogen in the plants, taken from the atmosphere:

SAMPLING	NITROGEN IN PLANTS TAKEN FROM THE AIR
	per cent
Two weeks before blooming	60.0
When blooms appeared	65.7
Full bloom	97.5
Mature	94.2

The largest percentage of total nitrogen in the plants was taken from the air at the last periods, almost the full amount coming in that way. There is no evidence of non-symbiotic fixation of nitrogen but even if it is assumed that some has occurred, most of the nitrogen in the plants at full bloom and at maturity must have been taken from the air.

At the earlier stages the proportion of nitrogen from the air was much lower. These results are quite in agreement with those secured with the alfalfa on the Carrington loam, where at maturity the total amount of nitrogen in the plants seemed to come from the air.

The calculations of the proportion of nitrogen in the tops taken from the air show that at the last three stages of growth all the nitrogen in the tops

came from the air and at the first period over three-fourths (77.1 per cent) was secured in that way. On the Carrington loam unsterilized, the total amount of nitrogen in the tops came from the air at every period, indicating the more rapid fixation of nitrogen in that soil. The results on the two soil types agree very well in showing the large amount of nitrogen which may be assimilated from the atmosphere. Even disregarding the possibility of non-symbiotic fixation of nitrogen which apparently occurred at least in the Carrington loam, it would seem that more than the nitrogen in the tops was taken from the air and at least a part of that in the roots was assimilated from the atmosphere.

Unfortunately, this series cannot be checked on sterilized inoculated soil on account of the failure of the crop under these conditions, perhaps due to physiological action which was noted in the case of the clover, an injurious effect of the sterilization.

DISCUSSION

It is quite impossible, of course, to draw any very definite or broadly applicable conclusions from the foregoing experiments. The results which have been secured must be considered applicable only to the two crops, red clover and alfalfa, and to the two soil types, Carrington loam and Miami fine sandy loam. They are also probably influenced to some extent by the fact that they were secured under greenhouse conditions and field results would not necessarily be exactly the same. They are indicative, however, of what may be expected in the field, and at least they serve to call attention to the variation in the proportion of plant growth of two legumes in tops and roots at different stages of growth and the differences in nitrogen content in tops and roots at the same stages, and under different soil conditions.

They indicate also how great the fixation of nitrogen from the atmosphere by individual leguminous plants may be under certain conditions. This throws some light on the question of how far legumes may be depended upon to keep up the nitrogen content of the soil, provided they are properly handled. In short, the data provide some evidence in support of previous assumptions, and show the impossibility of making definite statements now for all conditions. Further work along this line under different soil conditions is very desirable and the careful study of some of the many factors governing legume growth, inoculation and nitrogen fixation should lead to more rational ideas and better farming practices.

The indications from the experiments reported in the previous pages may be summarized here briefly in order to direct attention to the more salient points which they bring out.

In the first place, the relative proportion of the total plant growth in the tops and roots of red clover and of alfalfa at different stages of growth and under different conditions should be noted. With red clover on unsterilized Carrington loam, the percentage of total plant growth in the roots varied

from 29 to 34 being the greatest at the period when the blooms appeared. The percentage at maturity was 32. On the same soil sterilized and inoculated, it varied from 27 to 34 per cent, the higher figure again being found when the blooms appeared. At maturity, however, only 27 per cent was in the roots, indicating a variation due to the sterilization of the soil. In the latter case the percentage in the roots decreased from the time when the blooms appeared to maturity, while in the former it decreased to full bloom and then increased.

On the unsterilized Miami fine sandy loam, the percentage varied from 26 to 42, being the greatest at full bloom. There was a gradual increase up to that stage and a slight decrease at maturity. On the same soil sterilized and inoculated, the percentage varied from 32 to 51, being the greatest at the first two stages and gradually decreasing to 32 per cent at maturity.

Conclusions from these figures are very difficult to draw and probably the only safe deduction to be made is that the amount of total plant growth in the roots of red clover varies with the soil, with the inoculation and with the general growth conditions. The variations from 32 to 40 per cent of plant growth in the roots at maturity may indicate the variation which may occur on different soils in the field. The average (36 per cent) may be a fair estimate of the proportion of the red clover plant in the roots at maturity, when grown on unsterilized soil. Under the somewhat artificial conditions brought about by sterilization and inoculation, the average proportion was less, 29 per cent, varying from 27 to 32 on the two soil types. The effect of inoculation, as tested by the method used here, was insignificant, but the sterilization may have changed the effects which inoculation would otherwise occasion. The greatest development of roots of the red clover occurred when the blooms appeared in all but one case, whether or not the soil was sterilized, and in all but one case the smallest proportion of plant growth was in the roots at maturity.

With alfalfa on unsterilized Carrington loam, the proportion of total plant growth in the roots was the greatest at the stage when blooms appeared, 53 per cent, the lowest 2 weeks earlier, 14 per cent, and slightly less at the later periods. On the same soil, sterilized and inoculated the percentages were very similar; 14, at the first period, 44, 46, and 48 at the three later periods, the largest figure being found at maturity. On the uninoculated Miami fine sandy loam, the percentage varied from 24 to 55, being the smallest at the first period and gradually increasing up to maturity. The average percentage for the unsterilized soils, when the alfalfa was at maturity, was 52, and on the sterilized soil, 48; so that the effect of inoculation was apparently not beneficial at least to root development. Whether there was any retardation by sterilization which subsequent inoculation was not able to overcome cannot, of course, be determined from these tests. It is evident, however, that a much larger proportion, about one-half in fact, of the alfalfa growth was in the roots than was the case with red clover which showed 36 per cent. It

is evident also that the period of greatest development of the roots came between the period 2 weeks before the appearance of the blooms and when the blooms appeared, and there is indication that the percentage of total plant growth in the roots, increased as maturity approached.

Considering the nitrogen content of the tops and roots of the red clover and of the alfalfa, there are some deductions which should be drawn from the tests. With red clover on the unsterilized Carrington loam there was very little difference at the different stages of growth in the proportion of total nitrogen of the plant in the roots. It varied from 20 to 24 per cent, and was the smallest at maturity.

On the sterilized soil the results were very similar, varying from 19 to 26 per cent, being again the smallest at maturity and the greatest at the earliest stage of growth. On the unsterilized Miami fine sandy loam, the reverse of these results was secured, the percentage varying from 25 to 34, the greater percentage being found at maturity, and the smallest at the first stage. On this latter soil sterilized and inoculated, the smallest percentage was at maturity, 27, against 51 at the first stage. It seems evident from these results that the percentage of total nitrogen in the roots of red clover depends upon the soil conditions and may be widely different on different soils. When sterilized and inoculated, the results were also very different in the two soils, which might be attributed to the difference in the effect of sterilization. The effect of inoculation was not shown to be of any great significance on the development of root growth or on the increase of the element nitrogen in the roots. In fact there seemed to be a slightly smaller proportion of the total nitrogen in the roots at maturity where the soil was sterilized and inoculated, but this effect may be entirely the result of the sterilization. It may also be due to a greater growth of tops in proportion to roots brought about by the inoculation. As a matter of fact, the plant growth, tops and roots, was increased on the Carrington loam, but the tops were increased to a much greater extent than the roots on the Miami soil; however, the plant growth was less than on the unsterilized soil. This would emphasize the difference in the effect of sterilization on the two soils and indicate that on the Miami soil the sterilization was somewhat injurious to plant growth.

It should also be remembered that the efficiency of the inoculating bacteria may be very different in the two soils. The organisms in the Carrington loam were apparently not as vigorous as the cultures added after sterilization, while in the Miami soil, either the organisms were very efficient in the unsterilized soil or the sterilization proved so injurious that the cultures introduced did not have the best opportunity for development. The latter explanation seems the more tenable, especially in view of the fact that the plant growth itself was restricted.

On the sterilized, inoculated soils there seemed to be more nitrogen in the roots at the earlier stages of growth, which may be an effect of inoculation and of a consequent increase in nitrogen fixed in the roots earlier in the develop-

ment of the plant. At the later stages of growth, the greater development of the tops on the Carrington loam caused a greater proportion of the nitrogen to accumulate above ground. On the Miami soil, as has been noted, the sterilization reduced the plant growth and the percentage of nitrogen in the roots was reduced because of a pronounced reduction in root development. It is quite probable that the inoculation of red clover may make considerable difference in the proportion of total nitrogen present in the roots at different stages of growth. Unfortunately, the sterilization factor complicated the problem here, and no definite conclusions can be drawn. Tests are needed which will show comparative results on uninoculated soils; i.e., soils on which no nodules will be formed, with those obtained on soils abundantly supplied with vigorous bacteria. The inoculation factor is certainly of some importance in determining the relative nitrogen of tops and roots and in showing how much nitrogen will be left in the soil when the clover crop is removed.

It is interesting to note also that there was more nitrogen in the roots of the red clover at maturity on the Miami fine sandy loam than on the Carrington loam. Perhaps there is some relation here between soil type and nitrogen in the roots, the greater content or proportion of this element being found in the roots on soils lower in nitrogen and organic matter. There is another factor to take into account, however, and it will be recalled that the plant growth was much less on the soil poorer in nitrogen. Some relation between total plant growth and nitrogen in the roots may be all that these results should be considered to indicate. It is believed, however, that the type of soil and particularly its content of organic matter and nitrogen will prove an important factor in determining the proportion of nitrogen in the roots of red clover, and these results seem to confirm this idea. There may be exceptions to the indications from these experiments, but they seem rational and serve to call attention at least to the desirability of further tests along this line on widely differing soil types, types whose color and texture are very different.

Considering now the alfalfa, it will be noted that on the unsterilized Carrington loam the proportion of nitrogen in the roots varied from 13 to 44 per cent, being the greatest at maturity and the least at the first stage. Very little difference occurred between full bloom and maturity. When the soil was sterilized and inoculated the figures were very similar, varying from 16 per cent at the first stage to 42 at maturity. Very little difference is shown here between the results on the soil differently handled and treated. In both cases the greatest percentage was found at maturity and the figures were about the same whether or not the soil was uninoculated or sterilized and inoculated artifically. The soil evidently was supplied with very vigorous organisms and the inoculation did not prove of any effect on the root development, or else the sterilization retarded development to just the extent that the inoculation increased it, giving about the same results as on the uninoculted soil. This of course is mere speculation.

On the uninoculated Miami fine sandy loam the proportion of total plant nitrogen in the roots varied from 22 to 47 per cent, being the smallest at the earliest stage and the greatest at maturity; just as was the case on the other soil. The actual proportion of nitrogen in the roots at maturity was greater on this soil than on the Carrington loam which is exactly the same result noted on the clover. The experiment with alfalfa on the Miami soil, sterilized and inoculated, was not carried to completion because of failure of the crop and no comparison can be drawn as to the relative influence of sterilization and inoculation on the two soil types. It seems, however, from the results on the Carrington loam that sterilization and inoculation did not increase the proportion of nitrogen in the roots except at the early stage of growth, which is similar to the results secured with clover.

The amount of nitrogen in the roots of alfalfa at maturity was much greater than in the case of clover, averaging on the two unsterilized soils 46 per cent, while with clover the average was 27 per cent.

Again it seems that on the soil poorer in nitrogen and organic matter, there was a somewhat greater proportion of the total nitrogen in the roots, but the difference was not so pronounced with alfalfa as with clover. Apparently the soil type influenced the proportion of nitrogen in the roots of alfalfa just as was true with clover, and this may be a reflection of the relation between the amount of crop growth of tops and the characteristics, both physical and chemical, of the soil on which the crop is grown.

The amount of nitrogen taken from the air by inoculated red clover and alfalfa plants was considerable, according to the figures obtained in these tests. With red clover on Carrington loam unsterilized, 12.8 cgm. of nitrogen per plant was fixed at full bloom. On the Miami fine sandy loam 15.9 cgm. was fixed per plant at maturity. In both cases the amounts fixed gradually increased from the earlier stages of growth up to maturity. In the first instance the figure obtained at maturity was somewhat less than that at full bloom but no duplicate was secured; so it should not be considered definite. When the soils were sterilized and artificially inoculated the nitrogen fixed per plant at maturity amounted to 17.0 cgm. on the Carrington loam. On the Miami fine sandy loam, however, the fixation was less than on the unsterilized soil, amounting to only 5.9 cgm. per plant. Thus the indications from the results on the proportion of nitrogen in the roots of red clover are borne out by the evidence here that the sterilizing and inoculating of the Carrington loam gave an increase in nitrogen fixed while with the Miami soil the reverse effect occurred undoubtedly due to the sterilization exerting an injurious influence on the Miami soil.

It may also indicate that the bacteria naturally present in the Miami soil were more efficient than those in the Carrington soil, and the introduction of vigorous organisms artificially did not prove of any significance.

There is some difference in the amount of nitrogen fixed by the same legume on different soils, but the results do not permit of definite conclusions here.

They show the greatest fixation at full bloom on the richer soil while at maturity the poorer soil shows more fixation, but in the former case the figures are uncertain, not being duplicated. It seems justifiable, however, to conclude that the soil type bears a very important relation to the nitrogen fixed.

With alfalfa on the unsterilized Carrington loam the fixation of nitrogen increased up to 24.9 cgm. per plant at maturity. On the same soil sterilized and inoculated, the fixation was slightly less, increasing up to 20.3 cgm. per plant at maturity. On the Miami fine sandy loam the fixation increased up to 17.0 cgm. per plant at maturity—a smaller amount than that found on the Carrington loam. The reverse of the test with red clover seems to have occurred here and the greatest fixation on the unsterilized soil occurred on the richer soil. The reverse from the clover results also is true here when the fixation on the sterilized inoculated soil is compared with that on the unsterilized soil. Apparently the inoculation with artificial cultures did not prove of great value. Perhaps the sterilization of the soil proved a deterrent to as great bacterial action as occurred naturally.

Comparing the nitrogen fixed by the two crops it will be noted that the clover fixed less nitrogen than the alfalfa on the Carrington loam, unsterilized, but more than the alfalfa on the Miami soil. This may be explainable on the basis of more efficient alfalfa bacteria in the Carrington loam while the clover bacteria were the more vigorous in the Miami soil. It may also be due to differences in the physical or chemical conditions in the two soils which affect the bacteria from the various legumes differently.

It is apparent from these results that from 12 to 25 cgm. of nitrogen per plant may be secured from the atmosphere by red clover or alfalfa, but this amount, of course, will not all be added to the soil if the tops are all removed and only the roots left in the soil. How much of the total amount of nitrogen taken from the atmosphere by a crop of red clover or alfalfa will be left in the soil if the hay crop is removed? This question is one of considerable significance and the results obtained in these experiments indicate that with red clover on the unsterilized Carrington loam practically all of the nitrogen in the tops came from the air, when the blooms appeared, while at full bloom and later more than that was fixed, some nitrogen apparently being fixed in the roots. On the Miami fine sandy loam almost all the nitrogen of the entire plant came from the air, enough to supply all in the tops and a large part of that in the roots. On the sterilized and inoculated Carrington loam sufficient nitrogen was fixed to just about fill the need of the tops but none extra, while on the Miami soil sterilized and inoculated all the nitrogen in the tops at full bloom came from the air but not quite all at maturity. These results serve to emphasize the fact that sterilization caused some bad effects in the soil and the results secured must be considered abnormal.

With the alfalfa all the nitrogen in the plants at maturity seemed to come from the air but there was evidently some action by the non-symbiotic nitrogen fixers, the azofiers. Apparently, however, all the nitrogen in the tops and a

part of that in the roots came from the air. Similar results were secured on the same soil sterilized and inoculated, and on the Miami fine sandy loam. Unfortunately it was impossible to learn how much nitrogen was fixed by the azofiers but from general information it appears that the amount is insufficient to change materially the results secured here. It may be concluded that with clover and alfalfa on these two soils all the nitrogen in the tops came from the air and a part of that in the roots also was obtained in that way. Even allowing for azofication there may be some increase in nitrogen in the soil from growing inoculated legumes like red clover and alfalfa on soils of high or low nitrogen content, even when the hay crops are removed.

SUMMARY

From these experiments with red clover and alfalfa on Carrington loam and Miami fine sandy loam, the following conclusions may be drawn:

1. On the average, 36 per cent of the total plant growth of clover was in the roots at maturity on the unsterilized soils. On the sterilized soil the percentage was lower.

2. A larger proportion of the total plant growth of clover was in the roots at maturity on the soil poorer in organic matter and nitrogen. On the better soil there was greater total growth and a much greater growth of tops.

3. Over one-half the plant growth (53 per cent) of the alfalfa was in the roots at maturity on the unsterilized Carrington loam—slightly less (48 per cent) on the unsterilized Miami fine sandy loam. On the sterilized, inoculated Carrington, the percentage was slightly lower.

4. The percentage of total nitrogen in the roots of clover and alfalfa was greater on the soil poorer in organic matter and nitrogen.

5. On the average 27 per cent of the total plant nitrogen was in the roots of clover at maturity under natural soil conditions, while with alfalfa an average of 46 per cent of the total plant nitrogen was in the roots.

6. With clover there was a greater fixation of nitrogen on the poorer soil while with alfalfa the greatest fixation was on the better soil.

7. From 12 to 25 cgm. of nitrogen was fixed per plant by clover and alfalfa on untreated soils.

8. With clover on both soils unsterilized all the nitrogen in the tops and some of that in the roots came from the air. On the Miami soil a larger proportion of the nitrogen in the roots came from the air than on the Carrington.

9. With alfalfa all the nitrogen in the tops and some in the roots came from the air.

10. When clover and alfalfa are grown and the hay crops removed, there may be some gain in nitrogen in the soil, the amount of the increase of the element in the soil varying with the legume, the soil type, the inoculation and the general growth conditions.

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PRELIMINARY NOTE ON THE MICROBIOLOGY OF THE SOIL AND THE POSSIBLE EXISTENCE THEREIN OF INVISIBLE GERMS

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Everyone is familiar with the fact that very little is known about the bacterial flora of the soil (bacteria, yeasts and fungi) not only from the viewpoint of tabulation of the species existing therein, but especially from the viewpoint of its significance.

Accurate studies of soil bacteriology, moreover, are lacking for a number of reasons, as will appear later, not the least important of which is the fact that for a long time the hygienists have occupied the field in this matter, especially considering the presence of bacteria in the soil as a contamination. This has led particularly to the study of soil bacteria as a manifestation of the injurious action of pathogenic species.

At present, however, it is well understood that the microbic flora forms an essential element of the fertility of an agricultural soil and that the pathogenic germs *par excellence* of the soil, those of tetanus and malignant oedema, are probably only paratrophic forms of quite common meta and prototrophic germs, viz., those of the group of butyric acid bacteria closely related to the amylobacteria, all of these being forms that must have, even if we do not yet know its character or extent, an important share in that great process, the decomposition of the cellulose under the surface of the soil.

Now the other reasons may be reduced perhaps to a single one, viz., the vast uncertainty that surrounds everything that has to do with the fundamental methods of research. Therefore, he who wishes to make a serious study of the soil from the microbiological viewpoint must first serve an apprenticeship, not only in order to master the methods, but also in order to form a personal opinion of these, which frequently extends to a criticism of these methods and even to their rejection, or at least to a loss of confidence in them. And to indicate merely a few phases of the problem affected by these deficiencies it will be sufficient to mention:

A. *The uncertainties in the computation of the microbes in the soil*, because of which through a slight change of method, there is an enormous change in numerical result.

B. *The difficulties proceeding from the detrimental influence that the number and variety of microorganisms are capable, during development, of exerting upon each other.* This we have noted several times and particularly as a basis for a general

statement which we have made and which may be expressed thus: In the majority of cases the number of germs that can be successfully developed from the soil, by isolating cultures diluted with agar-agar and gelatine, is inversely proportional to the quantity of soil infusion employed for the determination. Thus, for instance, with 1/15 cc. of soil infusion there is generally obtained a larger number of germs than with 0.1 cc. One of a series of experiments, for instance, contains these figures:

a. In 23 analyses, 23 cases gave a greater development of germs from 0.02 cc. than from 0.1 cc.

b. In 23 analyses, 22 cases gave a greater development of germs from 0.04 cc. than from 0.1 cc.

c. In 34 analyses, one-half of the cases (17) gave a larger number of germs which developed from 0.04 cc. and in another half of the cases (17) a larger number was obtained with a 0.1 cc. sample.

C. *The influence of the methods employed for the isolation of the microbial species of the soil.* At present, it is clearly proven from the experience we have had for so many years that the examination of the soil for microbial species depends upon the method of isolation employed and especially upon the nutritive character of the medium selected. Agar-agar or gelatine (or even potatoes), however prepared, never gives anything different from the usual mesentericus, fluorescens, pigmented cocci, radiobacter (radiciformi ?), streptotriceae, molds, etc., and there are always in the soil, a few germs such as the *B. mycoides* which, if there are but a few individuals, are sufficient to hinder the development of all the other species.

And what should be done in case of all the other groups? For these we must always have recourse to selected enriched cultures which indicate absolutely nothing as to the actual relative number of germs, and hence as to the importance of these groups in the soil, not to say that even these selected culture methods are very frequently inadequate, hence the following difficulty:

D. *The inadequacy of the methods as applied to the isolation of the given species.* We have for instance devoted much time to isolation of the nitrifying bacteria and have convinced ourselves that quite correctly Winogradsky, Migula and Warington have regarded their presence as entirely salutary. Frequently on infecting with soil even eight to ten large flasks of selected nutritive liquids we have in none developed nitrite or nitrate bacilli.

E. *The enormous difficulty of sterilizing the soil completely.* Everybody is aware that the autoclave and the steam sterilizer of Koch, within normal time limits, are not successful in sterilizing the soil; neither is the hot-air sterilizer, nor roasting by direct heat.

This especially has been the reason why for some time we have been proceding cautiously in experimenting on the soil. It is indeed a standing rule of this institute to hold that no importance can be assigned to any microbe whatsoever in any medium whatsoever if we cannot repeat the phenomenon with the microbe in question, with a pure culture, and not only in the particular

medium of its own natural flora, but also without altering this meduim in its character and properties. We should consider that the soil is a complex chemico-physical structure. It contains colloids coagulable by heat. Moreover, it contains a circulating solution that may be considered *the true medium* of the soil in which microbes act, producing reactions and sustaining their influence.

I mean that since microbes form the *biological basis* of the complex system, it is clear that any sterilization, chemical or physical, must affect profoundly the capacities of a method so liable to falsify the results of any possible experiments.

F. *The lack of sensitiveness of certain chemical methods*, which, on the other hand, are the only ones at our command in order to understand the reactions produced by microbes, is also an obstacle. Of this we need give only two examples, viz., the lack of sensitiveness of methods for the determination of nitrogen which is necessary for the study of nitrogen-fixing organisms, and for which there has been found thus far nothing better than the fundamental method of Kjeldahl and its modifications, revealing only quantities that are calculable in a few milligrams; and the difficulty of the search for nitric acid in the presence of large quantities of nitrous acid, for which, even qualitatively, we possess nothing more sensitive than the very inadequate method of Lunge-Luvoff.

Wherefore, to sum up, we believe that we must be very cautious about regarding as fully known the *entire* micro-biological mechanism of certain soil phenomena such as those of nitrification and the fixation of nitrogen. It is insufficient for their interpretation to have succeeded in isolating from the soil germs which when placed in glass nitrify or fix nitrogen. It would be as if we should admit that in the soil there is alcoholic fermentation as an essential phenomenon simply because we can isolate from the soil yeasts which produce this process.

Hence all this obscures very much all that we know, not indeed concerning the biochemical action of many groups of germs, but certainly concerning their *real value* in the soil.

Furthermore, it is important not to forget that many of the theories concerning the soil processes are based upon phenomena which occur and are studied with germs isolated from the soil and allowed to act in artificial solutions which can certainly not be compared with the circulating solution.

It is therefore the inadequacy of the recognizable microbic flora in the soil to explain many biochemical phenomena that here occur, which has led us to seek the possible presence of an invisible, or rather an ultramicroscopic flora, or indeed to solve the question whether there exist invisible germs in the soil.

A direct method has been employed, that is, it consisted of taking fresh soil, mixing it with ordinary water in equal weights and allowing it to decant for 30 minutes, filtering the decanted liquid with the porous candles by means of the Gay-Lussac pump in the Chamberland apparatus. The liquid containing the supposed invisible germs would descend aseptically into Erlenmeyer flasks

containing culture liquids aseptic by test, in which the aforesaid microorganisms might produce the anticipated reactions.

About 50 cc. were filtered off which were mixed with 300 cc. of test liquid, and the 50 cc. were measured by a mark previously made with a diamond on the Erlenmeyer flasks.

It was the writer's intention to observe the principal reactions that are attributed to invisible germs, viz., putrefactive reactions (test for ammonia, indol and phenol), nitrite and nitrate reactions, and fixation of nitrogen. Naturally it was necessary to ascertain beforehand that the filtered liquid did not already contain the principles which it should produce.

From the researches made thus far with the soil of Gusserie Park of our own school (in the months of April and May) we have been able to deduce that:

1. The filtered infusion of soil (which we might also call circulating solution) does not contain indol but does contain ammonia and nitrites.

2. The results have been negative in an attempt to produce indol (tested with the familiar nitroso-indol reaction) from the nutritive liquid containing peptone. Needless to say, the same liquid unfiltered produced it abundantly as a result of the ordinary agents of putrefaction.

3. The sterile circulating solution did not cause any increase in nitrites beyond those which were present naturally in a liquid of the following composition:

Distilled water	100.00
Ammonium sulfate	0.01
Dipotassium phosphate	0.10
Magnesium sulfate	0.05
Sodium chloride	0.20
Ferrous sulfate	0.04

After sterilization, an excess of magnesium carbonate was added.

This last experiment was conducted in the following manner. To two Erlenmeyer flasks with 300 cc. of the aforesaid sterilized liquid was added 50 cc. of the sterile circulating solution. One of the two was then sterilized to serve as a control and the other preserved in a thermostat at 28° to 30°C. Two weeks later the contents were tested in nitrites and were found unchanged by the Gries reaction.

Clearly this is insufficient to disprove invisible germs. The experiments are still being conducted and surely will be interesting whatever their outcome.

THE COLORIMETRIC DETERMINATION OF SOIL IN A COLORED WATER EXTRACT

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Recently it became necessary, in connection with other investigations, to make a large number of nitrate determinations on soils containing varying and often excessive amounts of actively decomposing organic matter. The filtrates from the water extract of these soils were colored a deep brown, resembling a weak caramel solution. This color persisted despite attempts to remove it by additions of the usual decolorizing agencies. As it was necessary to make the determinations as rapidly as possible and because it has been shown (5) that the phenoldisulfonic acid method is extremely accurate in the absence of chlorides and sulfates (neither of which were present in sufficient amount to interfere), means were sought to decolorize the solutions in order that the nitrates might be determined colorimetrically.

Greaves and Hirst (6) in discussing the preparation of a clear filtrate state, "it would appear that the most likely flocculants are sodium, potassium and iron alum; ferric sulfate; lime; finely precipitated calcium carbonate and talc." They were working with soils containing alkalis in varying amounts and only normal amounts of organic matter. They found that their solutions could be clarified by the addition of 2 gm. of alum to the soil by filtering through a Pasteur-Chamberland filter or by centrifuging.

These flocculants, however, failed to decolorize the soil solutions obtained in this work despite the fact that the quantity added to the soils varied from 1 to 10 gm. Filtrations were made through Pasteur-Chamberland filters of medium fineness, through 3-inch alundum cones and through Büchner funnels using Whatman no. 31 discs of double thickness. As no trouble was experienced in obtaining a clear but highly colored solution the centrifuge was not used. Other agents—lamp black, bone black, powdered charcoal, animal charcoal, charcoal from blood and a commercial organic carbon Norit[2] reported by Bradley (3) were tried, but with the single exception of the latter all failed to decolorize completely. The Norit, however, was objectionable in that it contained a measurable amount of nitrates and the investigation was continued in the hope of securing an agent common to all laboratories.

[1] Contribution from Department of Farm Crops and Soils, Iowa State College, W. H. Stevenson, Professor in charge.

[2] Sample furnished by the Joseph Baker Sons & Perkins Company, Incorporated, White Plains, New York.

Attempts were then made to oxidize the organic matter by the use of potassium permanganate as described by Syne (7). After a thorough trial this method was discarded on account of a development of an interfering color in the subsequent evaporation to dryness. Bromine was then substituted for permanganate as an oxidizing agent and a colorless solution secured that dried in a satisfactory manner. The results, however, showed too low a nitrate content. When known amounts of nitrates were added to the colored solution before oxidizing it was impossible to recover over 25 per cent.

The next method tried was the aluminum reduction method of Burgess (4) as described in the official methods(2). These methods make no provision for soil nitrate determinations in a colored extract but state that the nitrates must be reduced to ammonia by aluminum in case more than 6 parts per million of chlorine are present. The inference is conveyed that in case of failure to secure a clear solution the reduction method is to be used. This method, aside from the time required for the determinations, was found to be objectionable on account of three exceedingly variable factors; namely, time for complete reduction, temperature at which reduction takes place, and nitrogen impurities in the aluminum foil.

A further search of literature revealed the fact that aluminum hydrate is recommended as a clarifying agent in water analysis (1). This may be prepared either by electrolizing ammonia-free water with the use of aluminum electrodes, or by precipitating the hydrate from an alum solution with ammonium hydroxide. In either case the precipitate must be washed by decantation until free of chlorine, ammonia and nitrates. This agent was found to remove all the color from the soil and manurial extracts used in this work, whether they were obtained from fresh or dried samples. As a result, attempts were made to perfect a method with this agent, using, as a basis of comparison, Norit as a decolorizing agent and the aluminum reduction method without decolorizing.

A large amount of aluminum hydrate was prepared from potassium alum; washed free of ammonia, nitrates and chlorides; allowed to concentrate as much as possible, and, after the volume of the concentrate had been determined, stored in a glass bottle for further use. At first definite amounts of the aluminum hydroxide solution were added directly to the soil solution before shaking. This procedure made the subsequent filtration difficult, as aluminum hydrate is primarily a decolorizing agent. The pores of the Pasteur-Chamberland filter were absolutely clogged. Better results were secured when 5 gm of precipitated calcium carbonate were used in addition. A decided improvement was made by adding 5 gm. of precipitated calcium carbonate directly to 100 gm. of soil, adding 400 cc. of water, shaking 15 minutes and filtering through a Pasteur-Chamberland filter. The color was then removed by adding a sufficient amount of aluminum hydrate filtering through a coarse filter paper and thoroughly washing the filtrate, directing the stream in such a manner as to free the gelatinous mass from the paper. The solution was then evaporated to dryness and the nitrates determined colorimtrically with phenoldisulfonic acid in the usual manner.

It was noticed that varying amounts of the agent were required to decolorize solutions of varying density. In searching for some material to give an indication of the proper amount to add it was found that a weak solution of caramel $C_{12}H_{18}O_9$ gave a color concentration very similar to that of the soil and equivalent amounts of hydrate would decolorize either. Accordingly, a 1 per cent caramel solution was prepared by dissolving 1 gm. in 100 cc. of water. A series of standards was then made by using 1, 2, 3, etc. cc. of this stock solution

TABLE 1

*Nitrate nitrogen recovered from colored soil solution variously treated**

SOURCE OF SAMPLE	COLOR OF SOIL SOLUTION IN TERMS OF STANDARD	NUMBER OF DETERMINATIONS IN AVERAGE	SOLUTIONS DECOLORIZED NITRATES DETERMINED BY PHENOLSULFONIC METHOD								SOLUTIONS NOT DECOLORIZED
			Decolorized with aluminum hydrate				Decolorized with norit				Aluminum reduction method
			Nitrates in soil	Nitrates added	Total nitrates recovered	Proportion of added nitrates recovered	Nitrates in soil	Nitrates added	Total nitrates recovered	Proportion of added nitrates recovered	Nitrates in soil
			mgm.	*mgm.*	*mgm.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*mgm.*
Soil from under manure pile..	9	4	25.51	10.00	35.21	97.0	26.42	10.00	30.30	38.8	24.23
Soil plus 36 tons of decomposing manure.....	2	4	2.05	10.00	11.85	98.0	2.00	10.00	2.69	6.9	2.37
Normal loam soil.....	Clear	4	1.51	10.00	11.56	100.5	1.96	10.00	11.44	94.8	1.74
Normal loam soil not treated with decolorizing agents.....	Clear	2	1.48	10.00	11.03	95.5					

* Results calculated on basis of 100 gm. of air-dry soil.

and building up to 100 cc. with distilled water. A scale ranging in concentration from 1 cc. of stock solution to 5 cc. will usually be sufficient for most soils, but manurial extracts will give a color concentration equivalent to a 15 or 20 cc. concentration. In this work it has been found that an aluminum hydrate solution containing the equivalent of $\frac{3}{4}$ gm. of potash alum is sufficient to decolorize an extract having a color concentration equal to (1) or 1 cc. caramel stock solution. Other concentrations will require 2 or 3, etc. times as much hydrate in regular rotation.

Results secured by the above method are submitted in table 1. These results were selected from a number of determinations made to test the efficiency of aluminum hydrate in decolorizing the soil solution for a determination of nitrates by the phenoldisulfonic acid method. The efficiency of Norit also is compared, while both are compared with the aluminum reduction method.

There is no question regarding the efficiency of either aluminum hydrate or Norit in successfully clarifying a soil solution without loss of nitrates. If, however, nitrates are added to the solution before decolorizing they are readily washed free from the aluminum hydrate precipitate, but are apparently partially absorbed by Norit. Since these resolutions were secured it has been found that the method may be modified to the extent that only one filtration is necessary. Five grams of precipitated calcium carbonate and 400 cc. of distilled water are added to 100 gm. of air- dry soil in 1-liter bottles. The soil is shaken 15 minutes, then allowed to settle 30 minutes. A 100-cc. pipette is then connected to suction pump with a long piece of tubing and an aliquot is quickly drawn off. A rubber stopper placed on the delivery tube of the pipette at such a height that the upper level of the solution only will be drawn off is of great assistance. The proper amount of aluminum hydrate is added to the aliquot, filtered through a coarse grade of paper and thoroughly washed. In this way the removal of color is a very rapid procedure.

SUMMARY

Soil solutions containing large amounts of soluble organic matter may be quickly decolorized by aluminum hydrate and the nitrate-nitrogen content determined colorimetrically by the phenoldisulfonic acid method.

The amount of aluminum hydrate necessary to decolorize the solution is quickly determined by comparison with a standard.

The method is accurate and exceedingly rapid. One filtration through a coarse quick filter paper removes all color.

A flocculating agent must be used to secure rapid results on fine-grained soils. Precipitated calcium carbonate is recommended.

No chemical reaction takes place that later may influence the development of color.

The decolorizing action is immediate.

Aluminum hydrate is prepared by dissolving 125 gm. of potash or ammonia alum in 1 liter of water. Add cautiously sufficient ammonium hydroxide to turn red litmus blue. Wash by decantation until free of ammonia.

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THE USE OF SILICA CRUCIBLES FOR THE DETERMINATION OF POTASSIUM IN SOILS

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Fusion of the finely ground material with a mixture of ammonium chloride and calcium carbonate in a platinum crucible of special design is perhaps the most distinctive feature of the J. Lawrence Smith method for the determination of potassium in rocks, minerals and soils. The shape and length of the crucible are such as to enable one to heat strongly the lower portion containing the charge while keeping relatively cool the upper portion to prevent loss of potassium through volatilization of its chloride. In the use of the J. Lawrence Smith crucible it is customary to insert it in an inclined position to any desired depth through the wall of a clay or asbestos board cylinder. The clay or asbestos board is sufficient protection against overheating the upper portion. This combination is entirely satisfactory only when one has good gas and comparatively few determinations to make.

In this state a large number of potash determinations are involved each year in connection with soil survey work. The enormous increase in the price of platinum made impracticable even several years ago the addition of new platinum crucibles to facilitate the analytical work. Our first substitutions were nickel crucibles of the J. Lawrence Smith type. Their first cost was much less. The life of the nickel crucible in this work, however, is comparatively short. Moreover, heating the crucibles in the manner recommended becomes somewhat tedious and cumbersome when the analytical work incident to the soil survey is reduced to a routine and energetically pushed. These reasons, together with our desire to reduce to a minimum the use of an unsatisfactory gas supply, induced us to try out for this work another substitute for platinum and finally to adopt a somewhat different procedure from what has been recommended heretofore for the fusions. The experimental work which follows justifies the modified procedure.

EXPERIMENTAL

The cracking of a nickel crucible of the J. Lawrence Smith type during a fusion and our inability to replace it at once led to the suggestion that several fusions be made in a silica crucible of the shape generally used for ignitions for comparison with fusions made at the same time of the same soils in a J. Lawrence Smith platinum crucible. We were somewhat curious to know how the

crucible would stand up under the treatment. The first fusion, made by direct heating with gas, was slaked at once in the usual manner and its washed insoluble residue digested with dilute hydrochloric acid. The fusion had been perfect. The potash content of this fusion was then determined, as was that of two subsequent fusions of other soils. The same crucible, a no. C1, vitreosil, porcelain shape—glazed, was used for the three fusions. A ½-gm. sample of soil was used in each fusion. The weights of potassium platinic chloride and corresponding percentages of potash obtained from fusions in the silica crucible and in the J. Lawrence Smith platinum crucible are stated in table 1.

After slaking there appeared to be a slight residue on the bottom of the silica crucible which required scraping for its complete removal. To find out whether

TABLE 1

Potash in soils by fusion in a platinum crucible of the J. Lawrence Smith type, and in a silica crucible of porcelain crucible shape

LABORATORY NO.	SOIL TYPE	FUSION IN J. L. S. PLATINUM CRUCIBLE		FUSION IN SILICA CRUCIBLE	
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O
		gm.	*per cent*	*gm.*	*per cent*
11435	Clay loam	0.0468	1.81	0.0470	1.82
11420	Wheatland silt loam	0.0333	1.29	0.0341	1.33
11408	Wapato silt loam	0.0397	1.54	0.0385	1.50

TABLE 2

Potash in soils by fusion of 1-gm. samples in silica and platinum crucibles of ordinary shape for 50- and 70-minute periods in a gas-fired muffle furnace

LABORATORY NO.	SOIL TYPE	40 MINUTE FUSIONS (P) PLATINUM (S) SILICA		70-MINUTE FUSIONS (P) PLATINUM (S) SILICA		FUSION IN J. L. S. PLATINUM CRUCIBLE	
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	K_2PtCl_6	K_2O
		gm.	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*
11407	Salem loam	0.1005 (P)	1.95	0.0995 (S)	1.94	0.1008	1.95
11429	Cascade loam	0.1054 (S)	2.04	0.1062 (P)	2.06	0.1066	2.06

this slight residue retained an appreciable amount of potash, fusions were next made in a gas-heated muffle furnace of 1-gm. samples of two other soil types—two in silica crucibles (no. C1) and two in platinum crucibles of the ordinary shape used for ignitions. The muffle was heated to dull redness previous to the insertion of the charged crucibles and was not permitted to get a great deal hotter at any time during the fusions. At the end of 40 minutes, one silica and one platinum crucible were removed. The remaining ones were removed at the end of 70 minutes. All fusions were then slaked and run for potash in the usual manner. The results are shown in table 2.

Upon examination of the crucibles after slaking, washing and drying, the platinum crucibles were found to be perfectly clean, and the silica ones to have

a slight residue adhering very tightly to the surface of the bottoms. Since potash in fusions made in the platinum crucibles checked a little more closely with the potash in fusions from the J. Lawrence Smith crucible than did potash from fusions in the silica crucibles, it would seem that a trace at least of potash was held back by the residues not readily slaked and removed by water. The possibility of losing potash by volatilization from the open crucibles was not overlooked, but apparently in the several fusions thus far made that loss was practically negligible. The closeness with which potash, in fusions thus far made in crucibles of the ordinary shape used for ignitions, checked with potash from fusions made in the J. Lawrence Smith platinum crucible, was remarkable. The silica crucible, in which four fusions had been made, appeared to be none the worse for the treatment received. A careful review of the situation brought us to the conclusion that although our experimental data at the time would support the proposal to substitute silica crucibles of simple design for the far more expensive J. Lawrence Smith platinum crucibles in soil analysis, the conditions under which they might satisfactorily and safely be used ought to be established. The advantages to be gained in time and labor by being able to make from eight to twelve fusions at once were sufficient inducements to carry the work farther than we originally intended. We determined to try out the adaptability of the electric muffle furnace for this work.

POTASH FROM FUSIONS MADE UNDER CONTROLLED CONDITIONS

It is evident that, on the one hand, the temperature of the crucibles must be sufficiently high to insure perfect fusion of their contents, and, on the other, that they should be kept below the temperature at which volatilization of potassium chloride is appreciable. Unless otherwise stated all fusions in silica crucibles mentioned hereafter were made in an electric muffle furnace equipped with a rheostat for temperature control. All fusions in the nickel and platinum crucibles of J. Lawrence Smith pattern were made with gas in the usual manner. Results from fusions in the platinum crucible of this type were accepted as the standard by which to judge all other results.

The furnace used is of Hoskins manufacture. It takes a current of 18.8 amperes at 110 volts. The first fusions were made without the use of a temperature indicator of any kind. The fusions were good but the results expressed in percentages of potash were much too low. It was evident that we had greatly exceeded the temperature at which volatilization of potassium chloride takes place. The muffle, 13 by 5 by 4 inches, has a circular opening in the back through which a thermo-couple was inserted when subsequent fusions were made so that its hot junction was about 4 inches from the front of the muffle and about $\frac{3}{8}$ inch above its floor. Readings were taken on a millivoltmeter and corresponding degrees of temperature from its calibration curve.

It was recognized that two factors, temperature and time, must be considered in making the fusions. It was impossible to eliminate completely the time element in determining the maximum temperature at which the crucibles must

be removed to avoid loss of potassium by volatilization. But, by so setting the rheostat arm as to permit of the maximum temperature aimed at being reached in the minimum of time, the influence of the time element was reduced to the minimum.

Temperature limits for fusions

In the first series, five charges of Willamette silt loam from the College Farm in duplicates of 0.5 gm. each in no. C1 silica crucibles, were placed in the muffle at room temperature. The current was then turned on. When the reading on the millivoltmeter reached 28, two crucibles were removed. As the reading on the millivoltmeter increased, two crucibles were removed for each increment of

TABLE 3

Effect of muffle temperature on potash determinations

A. WILLAMETTE SILT LOAM BY FUSION IN J. L. S. PLATINUM CRUCIBLE, ½ GM., K_2PtCl_6, 0.0781 GM., 3.03 PER CENT K_2O				B. NO. 11707, KIRBY GRAVELLY LOAM BY FUSION IN J. L. S. PLATINUM CRUCIBLE, 1 GM., K_2PtCl_6, 0.0762 GM., 1.48 PER CENT K_2O		
Reading on millivoltmeter	Time from turning on current	K_2PtCl_6	K_2O	Time from turning on current	K_2PtCl_6	K_2O
	minutes	*gm.*	*per cent*	*minutes*	*gm.*	*per cent*
28	80	0.0541	2.10	66	0.0490	0.95
		0.0567	2.20		0.0464	0.90
30	100	0.0767	2.97	77	0.0748	1.45
		0.0760	2.95		0.0746	1.44
32	117	0.0778	3.02	88	0.0770	1.49
		0.0768	2.98		0.0750	1.47
34	130	0.0715	2.77	104	0.0732	1.42
		0.0708	2.75		0.0722	1.40
36	160	0.0683	2.65			
		0.0664	2.57			

temperature corresponding to two points. The maximum reading was 36. The several fusions were then slaked and potash determined in the usual manner.

In the second series, four charges of soil no. 11707, Kirby gravelly loam, in duplicates of 1 gm. each, were treated in precisely the same manner as were the five charges of the first series. The maximum reading reached on the millivoltmeter, however, was two points lower. In this series of fusions the potash also was determined by the usual methods of procedure after slaking in water. Results for the two series are expressed in table 3 for comparison with results from fusions of the same soils in the J. Lawrence Smith platinum crucible. Figure 1 presents the same results graphically.

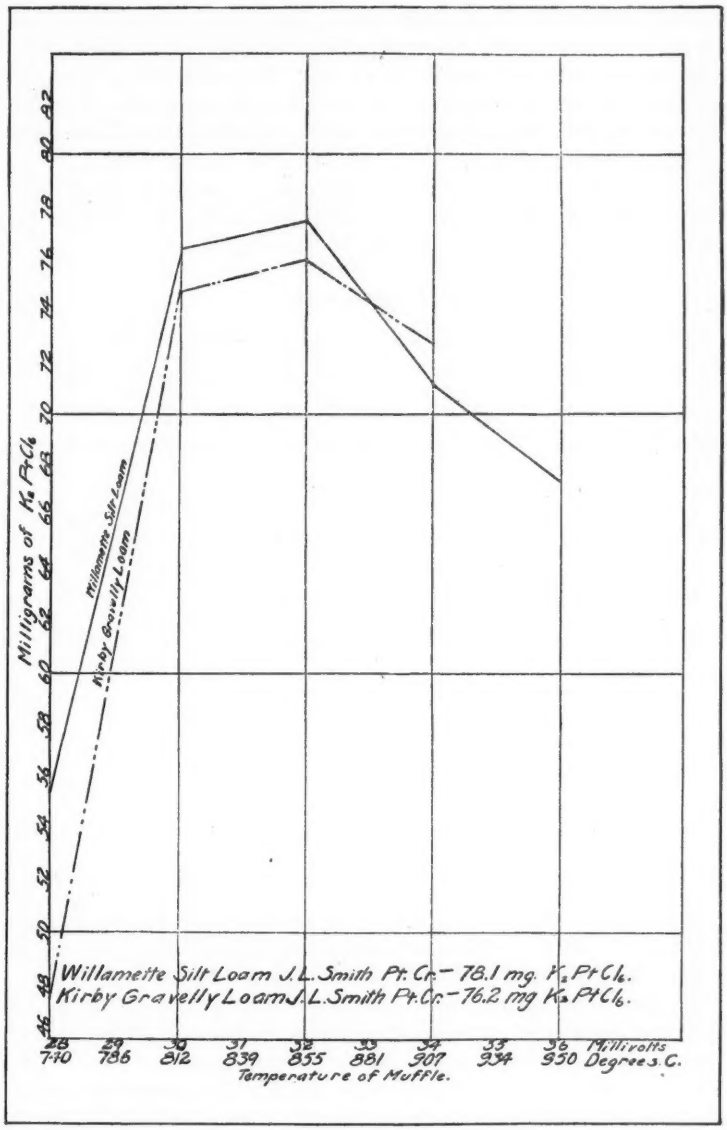

FIG. 1. EFFECT OF MUFFLE TEMPERATURE ON LOSS OF POTASH BY VOLATILIZATION

Fusions below reading 30 were noticeably imperfect; those above reading 30 were perfect, as one would judge them by examination of the residues insoluble in hydrochloric acid. Percentages of potash from fusions below reading 30 and above reading 32 were decidedly low. We concluded: (a) That to secure perfect fusions, in a reasonable time at least, the temperature corresponding to reading 30 on the millivoltmeter, 812°C., must be reached; and (b) that reading 32, corresponding to 855°C., should be the maximum aimed at, or, if the rheostat arm is set for a higher temperature, as perhaps it should be, the crucibles should be removed promptly when that reading is reached to avoid appreciable loss of potassium chloride by volatilization.

The time element

While the conclusions reached above are warranted from the data on the two soils used, it is conceivable that less danger of loss of potassium might be incurred and perfect fusions still obtained by fusing for a longer time at a lower temperature. We next determined how long the charges might be safely held at this lower temperature, 812°C.

TABLE 4

Effect of time of fusion on potash determinations; temperature constant at 30 millivolts, 812°C.; Willamette Silt loam, 0.5 gm.

TIME OF FUSION	K_2PtCl_6		K_2O	BY FUSION IN J. L. S. PLATINUM CRUCIBLE	
				K_2PtCl_6	K_2O
minutes	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*
60	0.0774	0.0760	2.99 2.95	0.0781	3.03
90	0.0767	0.0779	2.97 3.02		
120	0.0771	0.0781	2.99 3.03		
150	0.0747	0.0748	2.89 2.90		

The rheostat arm of the furnace was so set that the maximum temperature aimed at corresponded to reading 30 on the millivoltmeter. When that temperature was reached, four crucibles in duplicate, each charged with 0.5 gm. of Willamette silt loam, were placed in the muffle. The drop of 1½ to 2 points was quickly recovered and reading 30 maintained for 150 minutes. Beginning with 60, two crucibles were removed at the end of each 30 minutes thereafter. The fusions were then all slaked and their potash content determined with the results stated in table 4 and expressed graphically by figure 2.

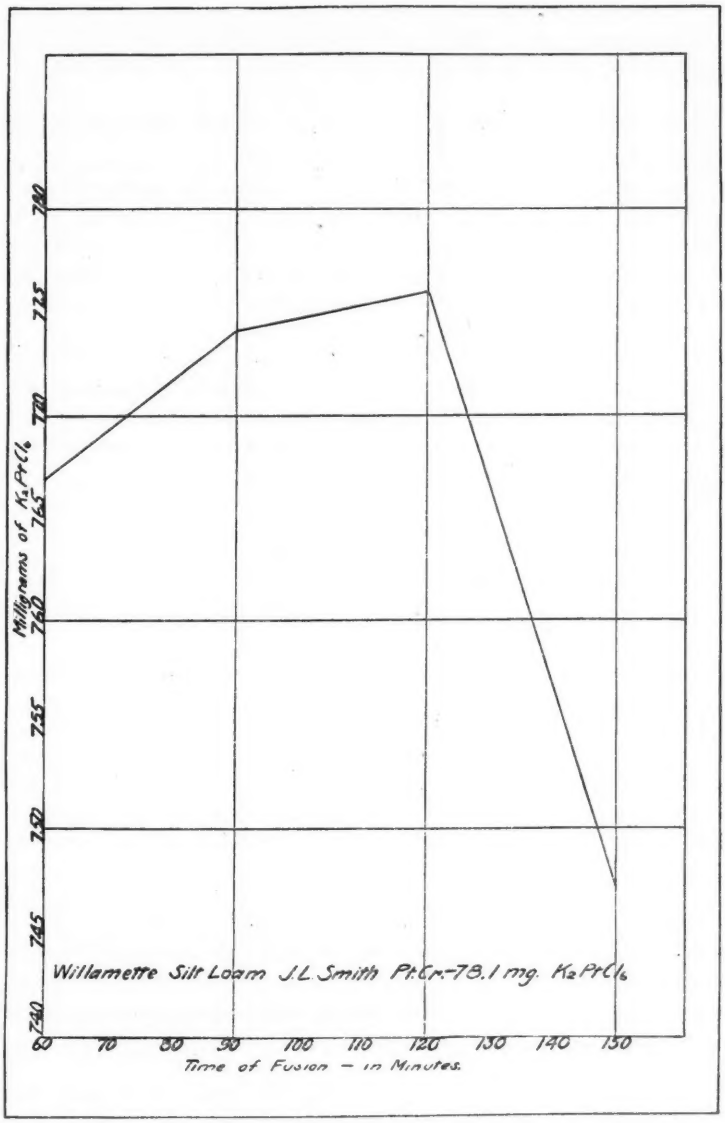

FIG. 2. INFLUENCE OF TIME OF FUSION ON LOSS OF POTASH BY VOLATILIZATION

It would seem that a fusion of at least 60 minutes at this lower temperature (812°C.) is necessary and that heating for another hour is not only safe but advisable to secure perfect fusions. Heating at 812°C. for more than 2 hours results in the loss of appreciable amounts of potassium by volatilization.

APPLICATION OF THE MODIFIED PROCEDURE TO ROUTINE ANALYSIS OF SOILS

Having established the temperature limits around which fusions of at least two types of soil in silica crucibles of simple pattern could be satisfactorily made, we selected a series of samples representing 12 soil types, surface and subsurface, as they occur in Josephine County for the determination of their potash content. Additional fusions of the 12 surface soils were made in both nickel and platinum crucibles of the J. Lawrence Smith type, but of the subsurface soils additional fusions were made in the J. Lawrence Smith platinum crucible only. Results from the fusions in silica crucibles were considered low or high as they varied from results secured from fusions made in platinum. One-half-gram samples were used in all cases. The procedure first outlined for the silica crucible was followed, inasmuch as there would be an appreciable saving of time if that procedure should prove to be generally applicable.

The charged silica crucibles, four in duplicate, were placed in the cold muffle with the rheostat arm of the furnace so set as to allow the maximum reading desired on the millivoltmeter being reached in the mimimum time. Approximately 1 hour and 20 minutes was required to reach reading 32. Promptly on reaching that reading the crucibles were removed from the muffle. The fusions were then cooled and leached and their potash content determined. Results for the 12 surface and corresponding subsurface soils, no two of the same type, are recorded in table 5.

A critical examination of table 5 will develop the fact that the percentages of potash of the 12 surface soils determined in the fusions in the three kinds of crucibles are in close agreement, with two exceptions. No. 11702 and 11705 fusions in silica do not check as closely as good work demands with the fusions in nickel and platinum. Since digestion with hydrochloric acid of the residues insoluble in water did not reveal unattacked mineral, we concluded, after rerunning them in precisely the same manner with practically the same results, that for these two soils the temperature of the muffle had been too high. For similar reasons the same conclusion was reached with reference to no. 11679 among the subsurface samples. These three soils were therefore rerun for potash by fusion for 2 hours with the reading on the millivoltmeter held at 30—corresponding muffle temperature 812°C.—with the results indicated also in table 5.

Judged by results secured with the surface soils alone, the tendency would seem to be for fusions in silica crucibles to run a trifle low in comparison with fusions made in the J. Lawrence Smith platinum crucibles. Judged by results secured on the subsurface soils alone the fusions in silica crucibles would seem to be a trifle high. It is possible that this difference between fusions of surface and

TABLE 5

Potash in soils by fusion of 0.5-gm. samples in silica crucibles with the electric muffle furnace

LABORATORY NO.	SOIL TYPE	FUSIONS IN SILICA CRUCIBLES		FUSIONS IN J. L. S. NICKEL CRUCIBLES		FUSIONS IN J. L. S. PLATINUM CRUCIBLES		DIFFERENCE BETWEEN FUSIONS IN SILICA AND IN PLATINUM
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	
			A. Surface soils					
		gm.	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*per cent*
11701	Colman gravelly sandy loam	0.0369	1.43			0.0364	1.41	
		0.0366	1.42			0.0366	1.42	+0.015
11702	Barron coarse sandy loam	0.0562	2.18	0.0585	2.27	0.0598	2.30	−0.105
		0.0569	2.21					
11702*	Barron coarse sandy loam	0.0598	2.30					
		0.0598	2.30					0.000
11703	Esterly gravelly clay loam	0.0368	1.43	0.0368	1.43	0.0370	1.44	−0.010
		0.0368	1.43					
11704	Columbia sandy loam	0.0348	1.35	0.0329	1.28	0.0348	1.35	−0.015
		0.0339	1.32					
11705	Aiken clay loam	0.0183	0.71	0.0204	0.79	0.0203	0.79	−0.115
		0.0164	0.64					
11705*	Aiken clay loam	0.0199	0.77					
		0.0192	0.74					−0.035
11706	Grants Pass clay loam	0.0180	0.69	0.0176	0.68	0.0176	0.68	−0.025
		0.0160	0.62					
11707	Kirby gravelly loam	0.0371	1.44	0.0391	1.51	0.0381	1.48	−0.030
		0.0375	1.46					
11708	Esterly gravelly loam	0.0306	1.19	0.0314	1.22	0.0317	1.23	−0.025
		0.0315	1.22					
11709	Kirby loam	0.0260	1.01	0.0267	1.03	0.0263	1.01	−0.015
		0.0253	0.98					
11710	Tokay clay loam	0.0170	0.67	0.0174	0.68	0.0174	0.68	+0.005
		0.0182	0.70					
11711	Holland coarse sandy loam	0.0561	2.18	0.0582	2.25	0.0575	2.22	−0.030
		0.0567	2.20					
11712	Colman loam	0.0513	1.99	0.0506	1.97	0.0514	1.99	+0.015
		0.0524	2.20					

TABLE 5—*Continued*

Potash in subsurface soils by fusion of 0.5-gm. samples in silica crucibles with the electric muffle furnace

LABORATORY NO.	SOIL TYPE	FUSIONS IN SILICA CRUCIBLES		FUSIONS IN J. L. S. PLATINUM CRUCIBLES		DIFFERENCE IN AVERAGES
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	
		B. Subsurface soils				
		gm.	*per cent*	*gm.*	*per cent*	*per cent*
11641	Colman gravelly sandy loam	0.0333	1.29	0.0324	1.26	
		0.0336	1.30	0.0321	1.25	+0.040
11643	Barron coarse sandy loam	0.0586	2.27	0.0576	2.23	
		0.0568	2.21	0.0573	2.22	+0.015
11646	Esterly gravelly clay loam	0.0325	1.26	0.0319	1.24	
		0.0325	1.26	0.0315	1.22	+0.030
11653	Columbia sandy loam	0.0274	1.06	0.0268	1.04	
		0.0270	1.05	0.0279	1.08	−0.005
11638	Aiken clay loam	0.0102	0.39	0.0094	0.36	
		0.0096	0.37	0.0093	0.36	+0.020
11673	Grants Pass clay loam	0.0189	0.73	0.0183	0.71	
		0.0194	0.75	0.0194	0.75	+0.010
11674	Kirby gravelly loam	0.0197	0.76	0.0195	0.75	
		0.0202	0.78	0.0195	0.75	+0.020
11678	Esterly gravelly loam	0.0278	1.09	0.0292	1.13	
		0.0280	1.10	0.0292	1.13	−0.035
11679	Kirby loam	0.0211	0.82	0.0230	0.89	
		0.0210	0.82	0.0223	0.87	−0.060
11679*	Kirby loam	0.0220	0.86			
		0.0212	0.83			−0.035
11684	Tokay clay loam	0.0097	0.37	0.0092	0.36	
		0.0097	0.37	0.0088	0.34	+0.020
11682	Holland coarse sandy loam	0.0556	2.16	0.0555	2.15	
		0.0544	2.12	0.0551	2.13	0.000
11693	Colman loam	0.0485	1.88	0.0482	1.87	
		0.0472	1.84	0.0471	1.84	+0.005

* Rerun at a lower temperature. See text.

subsurface soils is characteristic because of differences between surface and subsurface soils in their content of organic matter. Fusions in the silica crucibles did not check with each other as closely as fusions in the J. Lawrence Smith platinum crucibles. But, inasmuch as duplicate fusions in the silica crucibles check as closely as fusions in the J. Lawrence Smith nickel with fusions in J. Lawrence Smith platinum crucibles, we believe we are warranted in claiming for the silica crucibles, used as we have used them with the muffle furnace, at least equal dependability. Again, inasmuch as nothing is to be gained by carrying out the analytical work on the soils with greater accuracy than obtains in sampling soil types in the course of soil survey work, the substitution of the silica crucibles for both nickel and platinum is not, we believe, open to serious adverse criticism. The combined use of these crucibles and the electric muffle furnace, carefully regulated as to temperature, makes our potash determinations much less costly; the sacrifice in accuracy, if any, can be but trifling in comparison.

As many as 24 fusions have been made in one crucible, during which time it has lost in weight a total of 232 mgm. It is, of course more fragile than at the beginning of its use, but it is still good for many more determinations. We have not gotten longer service from our nickel crucibles of the J. Lawrence Smith type. Indeed so well have these crucibles stood up under the treatment given them that we thought it worth while to experiment still further with the idea of securing eventually a combination of electric heat and silica crucibles of the J. Lawrence Smith type fully as dependable as are the time-honored J. Lawrence Smith crucibles of platinum. The success attained is outlined in the concluding paragraphs of this paper.

For routine work in the analyses incident to the soil survey we are now using the electric muffle furnace and vitreosil crucibles, no. C6, with thoroughly satisfactory results. Briefly stated our procedure is as follows:

One-half gram of soil, ground to pass a 100-mesh sieve, is thoroughly mixed with 4½ gm. of a fusion mixture consisting of ammonium chloride and calcium carbonate in the proportion 1 to 8. When the charge has been transferred to the crucible, a hole is made in the mixture from top to bottom with a glass stirring rod close to the crucible wall. With the glass stirring rod, the fusion mixture is then tamped into this hole, causing it to spread out in a thin layer on the crucible bottom under the charge and finally to fill almost completely the hole itself. The crucible is then tapped lightly on the desk to distribute evenly the top of the charge. Thus prepared it is ready for the muffle. From 4 to 8 crucibles in duplicate make up a single run. They go into the muffle at room temperature and are removed promptly when reading 32 on the millivoltmeter (855°C.) is reached. The time required for fusion is approximately 1 hour and 20 minutes. When the crucibles are sufficiently cool, enough water is added to cover the fused mass in each and they are set aside for a couple of hours for slaking and digestion. Very frequently the fusions are timed so that slaking and digestion take place over night. Filtration and thorough washing of the digested material usually results in potash solutions of approximately 600 cc. From here on the procedure is practically that of the Association of Official Agricultural Chemists, the potassium being weighed in porcelain gooch crucibles as K_2PtCl_6.

USE OF J. LAWRENCE SMITH SILICA CRUCIBLES

The crucibles thus far tried out were made for us according to specifications. They are of transparent vitreosil, 10 cm. in length, 2 cm. in diameter at the top

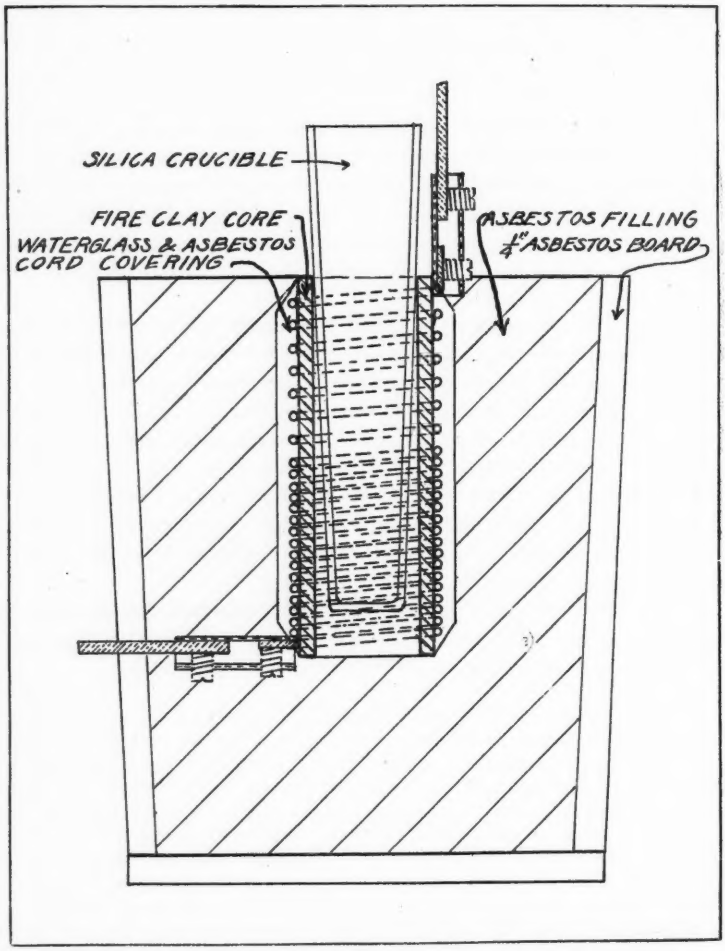

FIG. 3. CROSS-SECTION THROUGH AN ELECTRIC FURNACE ESPECIALLY DEVISED FOR USE WITH SILICA CRUCIBLES OF THE J. LAWRENCE SMITH TYPE

and taper to a diameter of 1.8 cm. at the bottom. Attempts to make fusions in them with the gas flame were not successful. The potash determinations in all cases were erratic and not dependable because of imperfect fusions. Obviously their use with the muffle furnace, if that were otherwise practicable, would

defeat the very purpose for which this type of crucible was designed. A simple electric furnace was finally devised that solved for us very satisfactorily the problem of heating these crucibles. In it the crucible stands erect. The lower end containing the charge may be heated to bright redness with no danger whatever of overheating the upper portion. In its crude state the furnace consists of a box of $\frac{1}{4}$-inch asbestos board 4 inches by 4 inches by 5 inches. The heating element is a discarded coil from an electric heater rewound to meet our purpose and protected from the air by several coats of a mixture of waterglass and finely divided asbestos fiber. This coil stands in the center of the box. The remaining space is tightly packed with asbestos fiber. From figure 3 it will be noticed that the crucible protrudes a full $2\frac{1}{2}$ cm. from the clay cylinder

TABLE 6

Potash determinations from fusions made in a silica crucible of the J. Lawrence Smith type with electric furnace heat and in a platinum crucible of the J. Lawrence Smith type, gas heated

LABORATORY NO.	SOIL TYPE	FUSION IN THE SILICA CRUCIBLE		FUSION IN THE PLATINUM CRUCIBLE		DIFFERENCE
		K_2PtCl_6	K_2O	K_2PtCl_6	K_2O	
		mgm.	*per cent*	*mgm.*	*per cent*	*per cent*
11738	Peat (surface)	14.0	0.543	14.0	0.543	0.000
11753	Whiteson silt loam (subsurface)	11.4	0.442	11.4	0.442	0.000
11757	Aiken silt loam (subsurface)	19.5	0.754	19.4	0.749	+0.005
11764	Aiken clay loam subsurface)	14.8	.0.574	15.2	0.589	−0.015
11771	Yamhill loam (subsurface)	29.8	1.155	29.9	1.160	−0.005
11773	Gale loam (subsurface)	31.9	1.235	31.0	1.207	+0.028

of the coil. The diameter of the cylinder should be such as to permit the crucible to rest with its top protruding not over 1 cm. It would be better still if the clay cylinder were replaced with a tapering tube with top and bottom diameters just great enought to allow the silica crucible to slide easily into it. The heating element is connected in series with a rheostat to insure for it a safe working temperature. A box of this size could easily be made to accommodate two heating coils, and a battery of, say, four or five similarly contrived furnaces would greatly facilitate potash determinations when fusions in crucibles of the J. Lawrence Smith type are thought to be necessary. Fusions in nickel or platinum crucibles, of course can be made in the same type of furnace, thus in any event freeing the analyst from troubles incident to an unsatisfactory gas supply.

The cost of the silica crucibles of the J. Lawrence Smith type is approximately the same as that of nickel crucibles of the same size and type. Our conviction is that they will last much longer. Future orders, however, will be for the glazed vitreosil. At present prices a battery of 12 silica crucibles can be secured for the price of one small platinum crucible. In the silica crucibles 1-gm. charges can be fused; a 0.5-gm. charge is the limit for the platinum. The silica crucibles, however, will give way much sooner than will the platinum.

In table 6 are shown analytical data for potash secured by the combined use of a transparent silica crucible of the dimensions previously given and the crude electric furnace illustrated in figure 3, and by fusion in a J. Lawrence Smith crucible of platinum. All fusions were of 0.5-gm. charges.

SUMMARY AND CONCLUSIONS

Nickel crucibles of the J. Lawrence Smith type are much cheaper than platinum and have been used very satisfactorily to a certain extent in soil analysis for the fusions incidental to potash determinations. They are, however, comparatively short-lived, and, if heated with gas in the manner customary with crucibles of this type, potash determinations are relatively slow and costly. Our desire to reduce to the minimum the use of an unsatisfatory gas supply, to lessen the cost and to speed up generally the analytical work involved each year in the soil survey of this state, led to a try-out of electric furnace heat and crucibles of silica for the fusions necessary in potash work.

We find that an electric muffle furnace and silica crucibles of the ordinary shapes used for ignitions can be used for the fusions with highly satisfactory results, provided certain limits of temperature are observed. To insure perfect fusions, the muffle must reach a temperature of 812°C., and, to avoid loss of potash by volatilization, its temperature must not exceed 855°C. Fusion at the lower temperature for approximately 90 minutes, and prompt removal of the crucibles from the muffle when its temperature reaches 855°C., give practically the same results. The first is probably the safer procedure; the second is quicker. A thermo-couple connected to a millivoltmeter, makes a very satisfactory temperature indicator. The number of fusions one can make at the same time is limited only by the size of the muffle. We find it practicable to make as many as eight in duplicate. Lessened expense follows from the substitution of electricity for gasoline gas, replacement of expensive platinum by relatively cheap crucibles and in the larger number of samples one analyst can handle in a unit of time. The sacrifice in accuracy is inappreciable for practical purposes.

A number of fusions of soil for potash have been made in silica crucibles of the J. Lawrence Smith type. When heated with gas the fusions were never perfect and the resulting figures for potash not at all dependable. When heated in an electric furnace of special design, fusions in these crucibles were perfect and resulting potash determinations were equally satisfactory with those made from fusions in platinum crucibles of the same type. For this combination of crucible and furnace careful regulation of temperature is not essential. The simple construction of the furnace described makes possible and practicable its substitution for an unsatisfactory gas supply when for any reason it is desired to use crucibles of the J. Lawrence Smith type of platinum, nickel or silica.